The coral-associated fauna and sessile benthic reef community of *Pocillopora* coral colonies following transplantation in Golfo Dulce, South Pacific, Costa Rica: Composition, dynamics and succession

Tesis sometida a la consideración de la Comisión del Programa de Estudios de Posgrado en Biología para optar al grado y título de Maestría Académica en Biología

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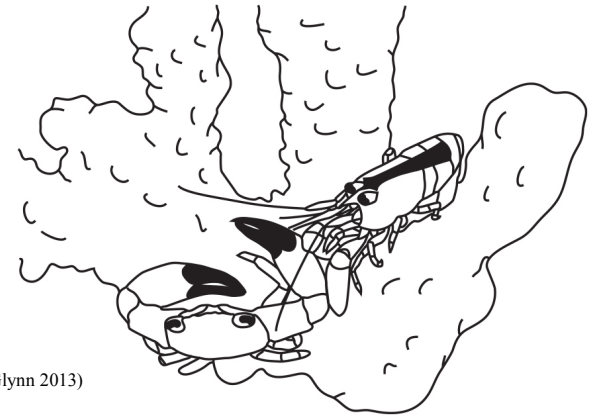
Agradecimientos


(Glynn 2013)

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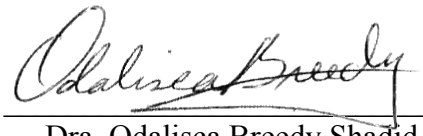

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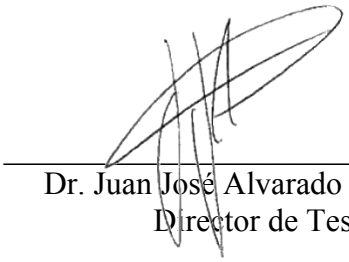

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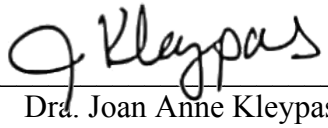

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Introducción

Los arrecifes de coral son ecosistemas asombrosamente diversos que brindan una amplia variedad de servicios ecosistémicos críticos para cientos de millones de personas en todo el mundo. Los arrecifes de coral son el segundo ecosistema con mayor diversidad biológica en cuanto a número de especies, solo después de las selvas tropicales, y albergan 32 de los 34 filos animales e innumerables otras especies de algas, bacterias y hongos entre otros. Estos organismos interactúan entre sí y con la estructura coralina en una compleja red de simbiosis, cada una de las cuales mantiene a las poblaciones cuidadosamente equilibradas y llevando a cabo procesos que permiten que el arrecife sobreviva y crezca. Esta gran diversidad es, en parte, el resultado de la complejidad estructural que brindan los esqueletos de coral y las constantes y leves perturbaciones naturales que experimentan los arrecifes, las cuales mantienen al ecosistema en un constante estado de sucesión donde no se tiende a alcanzar el equilibrio.

Esta perturbación constante también deja a los arrecifes vulnerables a cambios de fase, donde las condiciones ambientales y ecológicas pueden conducir al arrecife a una trayectoria de sucesión que favorece el crecimiento de coral y el arrecife, o favorece el crecimiento de algas, lo que puede resultar en el declive final del arrecife. A medida que las perturbaciones del ecosistema arrecifal se vuelven más severas y frecuentes debido al cambio climático y otros factores antropogénicos, como la eutrofización, el desarrollo costero y las pesquerías mal reguladas, los arrecifes de coral a menudo no se recuperan sin intervención. La restauración es una estrategia que puede utilizarse para fomentar la recuperación de este tipo de ecosistemas, evitando las fases de sucesión que no suelen ocurrir en ecosistemas gravemente degradados. El método de restauración de arrecifes más común es la jardinería y el trasplante de coral, que omiten el reclutamiento natural de coral, un proceso menos probable de que ocurra en arrecifes degradados.

La restauración arrecifal requiere no solo la recuperación de la estructura y marco coralino, sino de toda la fauna y flora asociadas, que llevan a cabo procesos críticos para el crecimiento y supervivencia del ecosistema. La criptofauna, toda aquella fauna asociada que ocupa los espacios entre las ramas de las colonias de coral, protege a los corales de factores estresantes, como depredadores o sedimentación. Su presencia se correlaciona positivamente con la supervivencia y resistencia de los corales después de las perturbaciones. Los peces son igualmente críticos para la salud de los arrecifes de coral, proporcionando servicios cruciales, como el control de organismos coralívoros o bioerosionadores, de algas y otros organismos bentónicos que compitan con los corales por sustrato.

Los corales ramificados del género *Pocillopora* fueron dominantes en los arrecifes de coral del Golfo Dulce, Pacifico sur de Costa Rica, hace más de 2000 años. Durante los últimos 1000 a 500 años, la cobertura de *Pocillopora* en el Golfo Dulce ha disminuido debido a factores naturales (inicialmente) y factores antropogénicos (más recientemente).

Actualmente, quedan muy pocas colonias de *Pocillopora* vivas en el golfo. Con tan pocas colonias restantes, la población de *Pocillopora* en el golfo podría haber experimentado el efecto Allee, una condición de una comunidad donde no hay suficiente densidad de población para sostener la reproducción sexual. Los corales masivos del género *Porites*, más resistentes a altas temperaturas y sedimentación, se han convertido en los corales dominantes en Golfo Dulce. Esto podría ser el resultado de un proceso de sucesión en el que *Pocillopora*, de crecimiento rápido, sucesión temprana y ramificación, estableció el arrecife de coral, pero finalmente fueron reemplazados por *Porites*, los cuales podrían tolerar mejor las presiones ambientales del golfo. Aunque la historia natural de los arrecifes de coral del Golfo Dulce sugiere que puede haber una trayectoria sucesiva hacia el reemplazo de *Pocillopora* por *Porites*, las colonias de *Pocillopora* que existen en el golfo han mostrado una notable resistencia a los factores ambientales estresantes. Con el fin de preservar el resto de *Pocillopora* en la zona y su fauna asociada, se está realizando un esfuerzo de restauración mediante el cultivo de fragmentos de colonias nativas en viveros y trasplantes.

Este estudio tuvo como objetivo monitorear el crecimiento y la salud de trasplantes de coral en el Golfo Dulce y los cambios en la estructura de la comunidad de fauna asociada en los mismos, así como la comunidad bentónica sésil posteriormente al trasplante de colonias de *Pocillopora*.

Métodos:

Treinta colonias de *Pocillopora* provenientes de viveros de coral, cultivadas a partir de fragmentos de colonias silvestres en el Golfo Dulce, se trasplantaron en un sitio de restauración experimental en julio del 2019. Simultáneamente, se instaló un parche control a la misma profundidad y área, sobre un sustrato similar pero que no contenía trasplantes de coral.. Los datos se recopilaron mensualmente durante 8 meses (de julio del 2019 a febrero del 2020) en el campo, utilizando equipo SCUBA. El proyecto se vio interrumpido por la COVID-19, lo cual imposibilitó la toma de datos a lo largo de un año completo.

<u>Capítulo I:</u>
Se observaron visualmente poblaciones de criptofauna y peces en los parches experimentales. Los cambios temporales en la comunidad de criptofauna asociados con los trasplantes se describieron en términos de abundancia, diversidad y composición de la comunidad en los 8 meses posteriores al trasplante. Los cambios en abundancia y diversidad se cuantificaron mediante una regresión lineal. Para cuantificar los cambios en la estructura de la comunidad, los meses de observaciones se agruparon en 3 “temporadas” y las abundancias de cada unidad taxonómica operativa (UTO) de criptofauna se transformaron en raíz cuadrada. Se realizó un análisis de similitud de las composiciones mediante un análisis no métrico escalar multidimensional (nMDS). Los cambios en la diversidad y abundancia de peces entre los meses iniciales y finales se compararon mediante una prueba t de Student, y la diferencia en la composición de la comunidad de peces entre el parche experimental y el control se describió con un nMDS.

Capítulo II:
Cada coral en la zona experimental, y clavo sin coral en el parche control, se fotografiaron mensualmente desde arriba, con plano cenital, a 40 cm, usando una cámara subacuática en un marco de PVC como referencia. La cobertura de área de corales y otros organismos bentónicos sésiles se extrajeron de imágenes fotográficas utilizando el software PhotoQuad. El área de crecimiento del coral (cm^2) en un plano horizontal se estimó trazando el área promedio de las colonias de *Pocillopora* cada mes con una línea de mejor ajuste usando una regresión lineal. La pendiente de esta línea fue la tasa de crecimiento de los trasplantes de *Pocillopora* en área (cm^2 mes^{-1}). La composición y sucesión de la comunidad bentónica sésil en los parches experimentales y de control se describieron trazando las áreas de cada UTO cada mes. Se realizaron regresiones lineales para cada UTO para determinar cómo cambiaban durante el período experimental posterior al trasplante. Se utilizaron nMDS y ANOSIM para medir el impacto de la presencia de los trasplantes de coral en la sucesión comparando las composiciones de la comunidad inicial y final de los parches experimentales y control.

Resultados

Capítulo I:
La abundancia y diversidad de la criptofauna aumentó significativamente durante el transcurso del experimento (regresión lineal: $p < 0.001$), y los decápodos simbiontes obligatorios como *Trapezia* spp., *Alpheus lottini* y *Harpiliopsis depressa* fueron las UTO más abundantes. La composición de la comunidad de criptofauna difería por agrupaciones "estacionales", siendo los peces del género *Scarus* que viven entre las ramas del coral el principal contribuyente a las diferencias. Aunque los resultados del nMDS y las pruebas asociadas no demostraron ninguna diferencia estadísticamente significativa en la composición de la comunidad de peces entre los parches (ANOSIM: $p = 0.074$), al final del período de estudio, la diversidad y abundancia de peces fue mayor en el parche experimental: 22 especies, con un promedio de 44.9 peces individuales observados en el parche experimental, y un total de 17 especies y un promedio de 33.1 peces individuales observados para el parche control.

Capítulo II:
Los trasplantes de coral experimentaron dos eventos de blanqueamiento, el primero, en agosto-septiembre de 2019 y el segundo, en enero-febrero de 2020. Al final de los 8 meses del experimento, 5 de las 30 colonias iniciales habían muerto y 20 se blanquearon, dejando 5 colonias sanas. Las colonias crecieron significativamente (regresión lineal: $p < 0.05$) en área de cobertura (cm^2) posteriormente del trasplante; cubriendo un 67% más respecto a su área inicial 8 meses después. Aunque las composiciones de la comunidad inicial y final de los dos parches no difirieron significativamente (ANOSIM $p > 0.05$), sí difirieron en la forma en que las áreas de las UTOs bénticas sésiles cambiaron en el área a lo largo del tiempo. En el parche experimental, las áreas de *Pocillopora*, algas costrosas coralinas (CCA) y cianobacterias aumentaron significativamente ($p < 0.001$) mientras

que el área de tapete de algas (turf) disminuyó significativamente ($p < 0.001$). En el parche control, las áreas de todas las UTOs bentónicas sésiles permanecieron constantes ($p > 0.05$).

Discusión

Capítulo I:
En cuanto a la criptofauna, los primeros organismos en ser observados fueron los crustáceos decápodos simbiontes obligados de las colonias de *Pocillopora*. Estos decápodos fueron seguidos por peces crípticos, organismos incrustantes y, finalmente, peces de arrecife más grandes. Se sabe que los tres decápodos más abundantes presentes en los trasplantes muestran una intensa agresión inter e intraespecífica, e inhiben que otros organismos se asienten en las colonias de coral, lo que puede explicar por qué comprendieron el 81% de toda la criptofauna observada. Esta agresión generalmente se extiende solo a otros miembros de su propia especie, limitando las poblaciones de estos decápodos a una pareja reproductora macho-hembra. Sin embargo, sin otras colonias de *Pocillopora* a las que trasladarse, los simbiontes decápodos podrían haberse agrupado más densamente en el parche de arrecife experimental. El aumento de la abundancia y diversidad de la criptofauna podría ser el resultado de la disponibilidad de más espacio entre las ramas del coral a medida que crecen las colonias. Asimismo, como se esperaba para los corales *Pocillopora*, la mayor diversidad y abundancia de peces observada en el parche experimental podría ser el resultado de la presencia de corales ramificados que promueven una mayor complejidad del ecosistema, y con ellos mayor disponibilidad de refugio y comida.

Capítulo II:
Al octavo y final mes del experimento, el 16.7% de los corales trasplantados habían muerto, el 66.7% estaban blanqueados y el resto, sanos. El primer evento de blanqueamiento observado pudo haber sido el resultado de las intensas lluvias en los meses de setiembre y octubre, provocando una mayor sedimentación sobre la zona de estudio y, por lo tanto produciendo, un mayor estrés en las colonias de coral. A su vez, una mayor cantidad de lluvias produce una disminución en la penetración de la luz, lo que afecta a las algas simbiontes. Así mismo, produce una disminución en la salinidad, lo que puede afectar procesos metabólicos, pues estos organismos no son osmoconformistas. El segundo evento de blanqueamiento en enero y febrero de 2020 podría haberse debido a temperaturas más altas en la superficie del mar, agravadas por la poca profundidad de los trasplantes y la lenta circulación de las aguas en la zona de estudio. Así mismo, en época seca el agua es mucho más clara, lo que aumenta la incidencia de rayos UV, que pueden contribuir al proceso de blanqueamiento. El aumento en la cobertura de coral y ACC, y la disminución en tapetes de algas en el parche experimental podría deberse a los herbívoros atraídos por la presencia de los trasplantes, posiblemente incluyendo los peces observados en el Capítulo I. El aumento de cianobacterias en el parche experimental podría ser el resultado de temperaturas más altas y puede haber sido un factor en la muerte de colonias.

Conclusiones

Posteriormente al trasplante, el ecosistema en y alrededor de las colonias de coral cambió a través del tiempo. A pesar de los dos eventos de blanqueamiento, los corales crecieron significativamente en términos de área, y la abundancia y diversidad de la criptofauna aumentó, a la vez que disminuyó la cobertura de tapetes de algas alrededor de las colonias. En comparación, el parche control, que carecía de trasplantes de coral, tuvo una menor diversidad y abundancia de peces de arrecife, y la cobertura de ACC, tapetes de algas y otros organismos bentónicos sésiles se mantuvo constante. Esto sugiere que, de aumentarse la cantidad de trasplantes, la restauración a través de la jardinería y el trasplante de coral puede cambiar la trayectoria de sucesión de un arrecife para apoyar el crecimiento de este y la abundancia y diversidad de la fauna asociada, con los procesos ecológicos que ellos proporcionan.

Esta información es útil para informar futuros esfuerzos de restauración sobre qué esperar en la sucesión temprana de la comunidad bentónica sésil, en torno a los trasplantes de coral. Se pueden hacer recomendaciones para futuros estudios y esfuerzos de restauración en el Golfo Dulce con base a los resultados de estos experimentos. Las colonias deben trasplantarse a una profundidad superior a los 2-3 m para minimizar así la radiación solar perjudicial, que puede contribuir al blanqueamiento y muerte de los corales. Los resultados también sugieren que puede ser útil trasplantar colonias de *Pocillopora* juntamente con organismos pioneros que facilitan el asentamiento y crecimiento de otras especies de coral, al fomentar procesos ecológicos críticos. Para futuras investigaciones, sería útil tener un mayor número de parches más pequeños, que podrían monitorearse a escala de una comunidad de coral o arrecife, además de los experimentos centrados en colonias a pequeña escala de este estudio.

Chapter I:

Change in the composition of fauna associated with *Pocillopora* spp. corals following transplantation in Golfo Dulce, South Pacific Costa Rica

Master's thesis of:

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Abstract:

The associated fauna comprise the majority of the diversity of a coral reef and carry out ecological functions essential to the reef's survival. Coral reefs, along with their associated fauna are in decline globally. As threats from climate changes and severe reef disturbance increase, coral reef restoration through coral gardening and transplantation are popular interventions. *Pocillopora* harbor the most associated fauna of any coral genus. To preserve *Pocillopora* colonies and their associated fauna in Golfo Dulce, Costa Rica, a reef restoration initiative is underway. Studying the succession of the associated fauna following coral transplantation is critical to understanding how a reef recuperates its ecological functions in the context of reef restoration. To understand the processes of succession of associated fauna in a coral reef, this project describes the abundance, diversity, and changes in the community of cryptofauna and fish associated with *Pocillopora* colonies following transplantation. An experimental patch of 30 nursery-grown *Pocillopora* colonies were transplanted and a control patch containing no colonies were monitored for 8 months following transplantation in Golfo Dulce. Cryptofauna and fish were observed visually using SCUBA. Temporal changes in abundance and diversity were quantified with linear regression. Non-metric multidimensional scaling (nMDS) was used to visualize changes in the community structure, using abundances of each operational taxonomic unit of cryptofauna across three "seasons". Change in the diversity and abundance of fish between initial and final months was described with t-tests and the difference in the fish community between the experimental and control was described with nMDS. The abundance and diversity of cryptofauna increased significantly over the course of the experiment, obligate simbiont decapods were the most abundant. The composition of the community of cryptofauna differed between "seasonal" groupings with fish in the genus *Scarus* as the main contributor to any differences. Although not statistically significant, the diversity and abundance of fish was greater in the experimental patch containing the coral transplants. Following transplantation, crytofauna flourished. The increase in abundance and diversity of cryptofauna may reflect availability of more space between coral branches as colonies grow. A greater diversity and abundance of fish observed in the experimental patch could also result from greater complexity due to the presence of branching corals. The composition of the crytofauna communities was generally consistent with the literature; however, the relatively high density of decapod symbionts suggests that without other *Pocillopora* colonies to move to, symbionts crowded despite their own aggressive tendencies. Coral-associated fauna provide critical ecological services to the reef. This study informs expectations regarding the critical processes of succession and dynamics of the associated fauna in the context of coral reef restoration.

Key words: cryptofauna, coral reef restoration, succession, eastern tropical Pacific

INTRODUCTION

Second only to tropical rain forests as the most biologically diverse ecosystem in terms of number of species, coral reefs are the most diverse in terms of higher taxonomic classification. Coral reefs harbor 32 of the 34 animal phyla compared to 12 that live in terrestrial environments (Reaka-Kudla et al. 1997, Stella et al. 2011). The extraordinary biodiversity of coral reefs can be attributed to the structural complexity of the corals and the framework that they construct. This structural complexity corresponds to a great diversification and quantity of ecological niches that, in turn, attract a large diversity of fauna (Stella et al. 2011). The cryptofauna, the animals that live in between the branches of the coral and the reef structure, are the most diverse but often overlooked reef occupants (Stella et al. 2011, Glynn 2013, Cortés & Reyes-Bonilla 2017). Many, if not most species of cryptofauna are symbiotic with corals, depending on the corals for protection, food, and reproduction (Abele & Patton 1976, Castro 1988, Stella et al. 2011). In return, cryptofauna carry out critical ecological processes within the reef ecosystem such as the protection of coral colonies and nutrient cycling (Stella et al. 2011).

Given the importance of coral reefs as centers of marine biodiversity, a great number of fish species have an ecological association with, or dependence on, coral as shelter, as direct or indirect food sources, and as sites of reproduction. Some fish live in closer association with the corals than others, ranging from cryptic species living within the branches to pelagic species that rely on food sources derived from reefs (Reaka-Kudla 1997). The reef fish, in turn, provide a variety of critical services to the reefs they inhabit (Hixon & Bronstoff 1996, Hixon 1997, Burkpile & Hay 2010). Herbivore fish and invertebrates graze on algae and reduce its coverage so that the algae do not compete with corals for space and resources (Hixon & Bronstoff 1996, Burkpile & Hay 2010, Humphries et al. 2014, Hughes et al. 2017). Invertebrate-eating fish protect the colonies from corallivorous invertebrates, and predatory piscivorous fish maintain healthy balances of reef species by controlling the populations of coral predators and bioeroding herbivores (Hixon 1997).

Environmental and biological factors affect the diversity, abundance, and species composition of coral reef ecosystems. Corals host a variety of cryptic organisms, often with species of symbionts that live exclusively on particular species of corals (Abele &

Patton 1976, Sin 1999, Stella et al. 2010, 2011). More complex, branching corals have the greater abundance and diversity of associated fauna than massive or encrusting species, with corals of the genus *Pocillopora* harboring the greatest number of associated species (Stella et al 2011). One of the most important contributing factors is the health of the coral itself. Live corals have greater diversity and abundance of fish than dead corals (Bel & Galzin 1984). Bleached corals have less abundance and fewer species of cryptic fauna than healthy corals (Tsuchiya 1999). On dead coral, there is an initial increase in biodiversity as boring invertebrates colonize the exposed carbonate skeleton, which is unprotected by the coral's stinging polyps (Enochs 2012). This diversity is not sustained, however, as eventually the skeleton is eroded away, and with it the structural complexity that sustains the biodiversity of a living coral reef (Enochs & Manzello 2012a).

The best sites for coral reef restoration are where corals previously flourished and are unlikely to settle and grow under the current marine conditions of the site (Rinkevich 2005). Likewise, the best candidates for species to be introduced are those that might not colonize the restoration area unassisted (SER 2004). *Pocillopora* may be thought of as a pioneer species and is a good candidate in that their branched skeletons create the conditions that allow other organisms to colonize an area where they previously would not be able (Clark & Edwards 1994, Darling et al. 2012). Coral restoration is essentially a facilitation and acceleration of natural successional processes through the reintroduction and management of key species (Young 2001, Walker et al. 2007, Horoszowski-Fridman et al. 2015, Horoszowski-Fridman & Rinkevich 2017).

The timing of recruitment of the associated fauna onto a coral colony appears to also be of importance for the reef's survival and health. For example, it is known that some cryptic fauna are recruited to corals quickly and begin to reduce the effects of sedimentation and provide protection from predation (Stewart et al. 2006), which are particularly important for juvenile corals compared to larger, more mature corals (Toh et al. 2014). In laboratory settings, juvenile host corals have higher survival rates when colonized with juvenile *Trapezia* spp., which offer the host coral protection from predation (Rouzé et al. 2014). However, it is not known when and in what order cryptic fauna are recruited to coral transplants.

Although non-coral animals such as fish and invertebrates make up the majority of a reef's biomass and diversity (Stella et al. 2011), most studies addressing the success of a restoration effort focus on the growth and survival of the corals themselves (Stella et al. 2011, Ladd et al. 2019). While the corals are clearly critical to form the basic structure of coral reefs that allows them to harbor such great biodiversity, the effect of reef restoration on the community structure of coral associated animals is relatively understudied by comparison. Most of the available literature on the succession of fish following restoration efforts focuses on non-living artificial reefs (Russel 1975, Santos et al. 2011, Becker et al. 2017). Similarly, studies concerning the succession or community structure of cryptic fauna on reefs have used non-coral substrates (Breitburg 1985) or the collection and destruction of coral colonies (Enochs & Hockensmith 2008, Enochs et al. 2011, Enochs & Manzello 2012b).

Given the importance of reef-associated animals to the health and survival of the coral, the ecosystem services provided by the associates, and the wide-ranging impacts of reef communities on the ecology of the oceans, more research on the effects of reef restoration on coral-associated animals is essential. Ecological restoration is considered to be successful when it can regain its full complement of native species and recuperate ecological processes essential for the long-term persistence and self-sustainability of the reef (SER 2004). These processes, such as symbioses, herbivory, reduction of competition, increase resilience and resistance of coral reefs to disturbance, anthropogenic or otherwise. Thus, ensuring these processes are taking place within the restored site is a vital step towards restoration success. The process of ecological succession is a good indicator that an ecosystem is on a healthy trajectory towards restoration (Walker et al. 2007).

In order to understand the processes of succession of the cryptofauna in a coral reef of the Eastern Tropical Pacific (ETP), this project aims to: 1) Describe the community of cryptofauna living in the coral transplants; 2) monitor changes in the community of cryptofauna in *Pocillopora* colonies in the 8 months following transplantation; and 3) explore changes in the reef fish community following transplantation. It was hypothesized that the abundance and diversity of cryptofauna would increase over time and obligate symbionts would be the first organisms recruited

to the colonies, followed by facultative symbionts. It was also hypothesized that the abundance and diversity of fish would be greater in an area with living transplanted coral than without it. The data collected for this project will be used to assess temporal patterns in the recruitment of reef-associated animals to the nursery-grown out-plants. Monitoring these patterns and relationships will increase the understanding of coral reef ecosystems, one of the most critical ways to combat the ecological crisis of coral decline (Bellwood et al. 2004). Locally, understanding the interrelationships between fauna and coral, the patterns of biodiversity, and succession roles in the restoration project in Golfo Dulce will provide key information for understanding success and for planning future restoration projects.

MATERIALS AND METHODS

Study area: Golfo Dulce is a narrow-mouthed embayment (8°27'–8°45'N, 83°07'–83°30'W) oriented northwest to southeast between the Osa Peninsula and the southern Pacific coast of Costa Rica (Fig. 1). The Gulf has an approximate length of 50km and width between 10 and 15km and covers an area of 680km^2 with a maximum depth of 200m. The average yearly rainfall in Golfo Dulce is 4,000 to 5,000mm and temperatures range from 18º to 35ºC with an average of approximately 26.5ºC. The dry season lasts from December to March with an average rainfall of 100 to 160mm per month. The wet season peaks in October with an average monthly rainfall of 800mm (Cortés 1990, Quesada & Cortés 2006). For the purposes of this study, data collection months, were grouped by "seasons" that roughly correspond to seasonal ranges in Golfo Dulce as decribed by Cortés (1990). The "Wet" season includes September, October, November & December, the "Dry" season includes January, February and March, and the "Transition" season includes April, May June, July & August. During the period of this study (July 2019 – June 2020), there was a total rainfall of 3687mm based on meteorological data from the Fundación Neotrópica station of the Instituto Meteorológico Nacional, near Golfo Dulce (08º42'02.9"N, 83º30'49.4"W, at 80m above sea level)(Figure 2).

Geographically and ecologically, Golfo Dulce is part of the ETP marine biological province (Glynn et al. 2017). It is often referred to as a tropical fjord due to its anoxic deep waters and bathymetry (Cortés 1990, Wolff et al. 1996, Hebbeln & Cortés

2001). The anoxic waters prevent the energy contained in detritus that sinks to the depth of the gulf to be recycled back into higher trophic levels of the ecosystem (Wolff et al. 1996). These unique conditions result in a community structure of fish and invertebrates that differ significantly from other marine ecosystems of the Pacific coast of Costa Rica (Alvarado et al. 2014).

Transplantation site: Punta Bejuco reef (8°43'39"N, 83°24'30"W) (Fig. 1) was selected as the transplantation site for the *Pocillopora* colonies that were cultivated in the nurseries in Nicuesa. These nurseries form part of a restoration initiative that started in 2016 as a project of the non-governmental organization Raising Coral Costa Rica and the Center for Research in Marine Science and Limnology (CIMAR) of the University of Costa Rica (UCR) (Villalobos 2019). Punta Bejuco consists of three reefs with steeply sloping edges separated by channels of sand. The reef substrates are mostly made up of dead *Pocillopora* and *Psammocora* rubble. The predominant live coral species are *Porites lobata* (Cortés, 1990) and *Porites evermanni* (Boulay et al. 2014). No live colonies of *Pocillopora* have presently been encountered in Punta Bejuco, but the presence of live *Pocillopora* can be inferred from coral skeletons (Cortés, 1990). *Pocillopora* have low rates of natural recruitment in Golfo Dulce (Villalobos 2019), and small colonies are more likely to die as a result of predation (Toh et al. 2014) and competition with algae (Kuffner et al. 2006). Cultivation of *Pocillopora* in coral nurseries allows colonies to reach a size that is more likely to survive once they are transplanted at a restoration site.

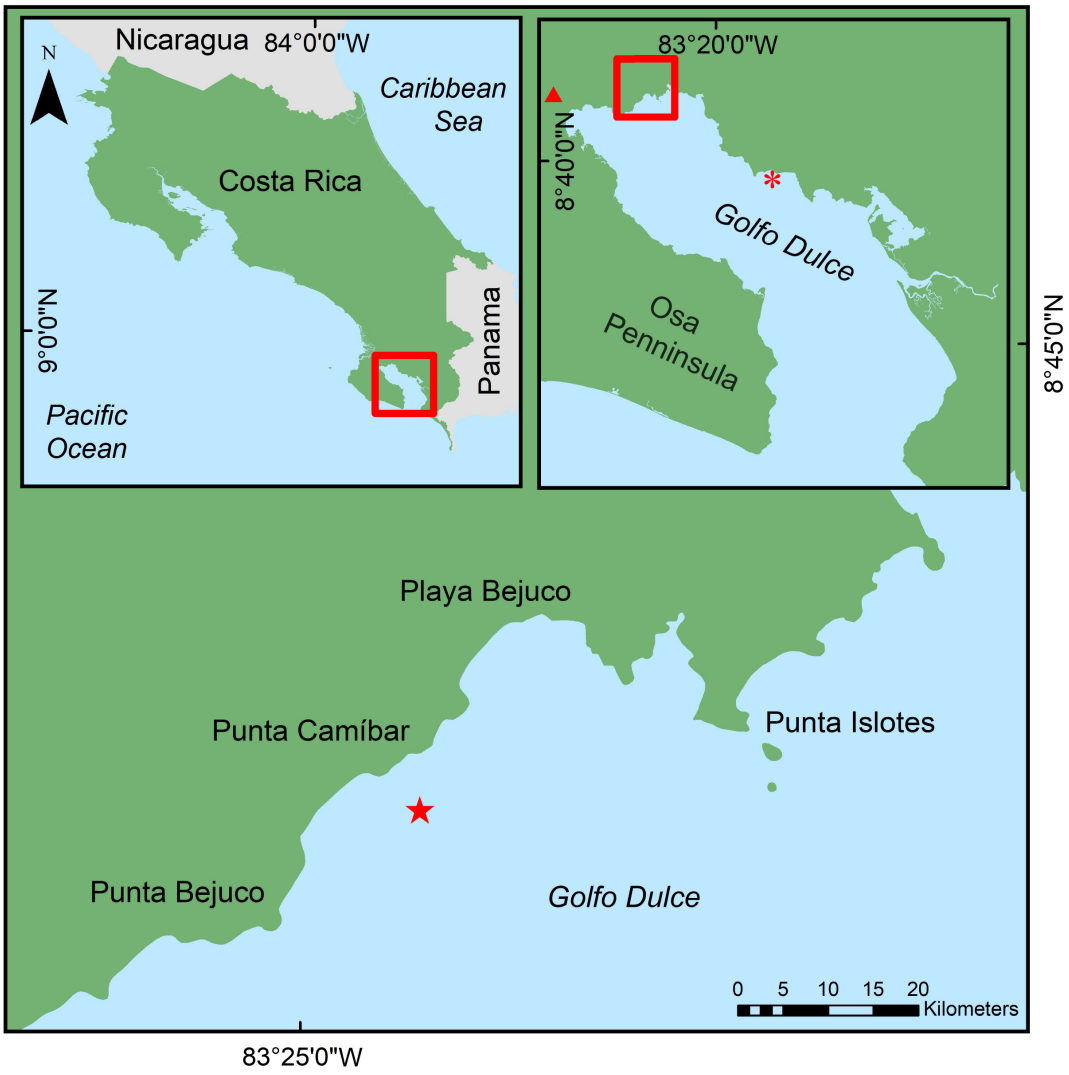

Figure 1. Location of Golfo Dulce within Costa Rica. Punta Bejuco (transplantation site) is marked with a red star and Nicuesa (coral nursery), is marked with a red asterisk. The red triangle in the top-right quadrant marks the meteorological station (Figure 2).

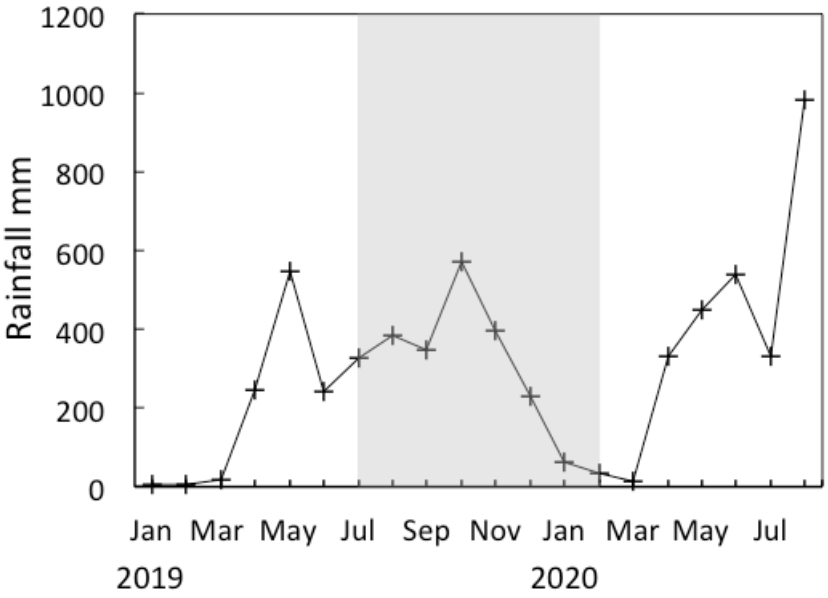

Figure 2. Rainfall (mm month^{-1}) data from the Instituto Meteorológico Nacional de Costa Rica, near Golfo Dulce (08º42'03"N, 83º30'49"W, at 80m above sea level).

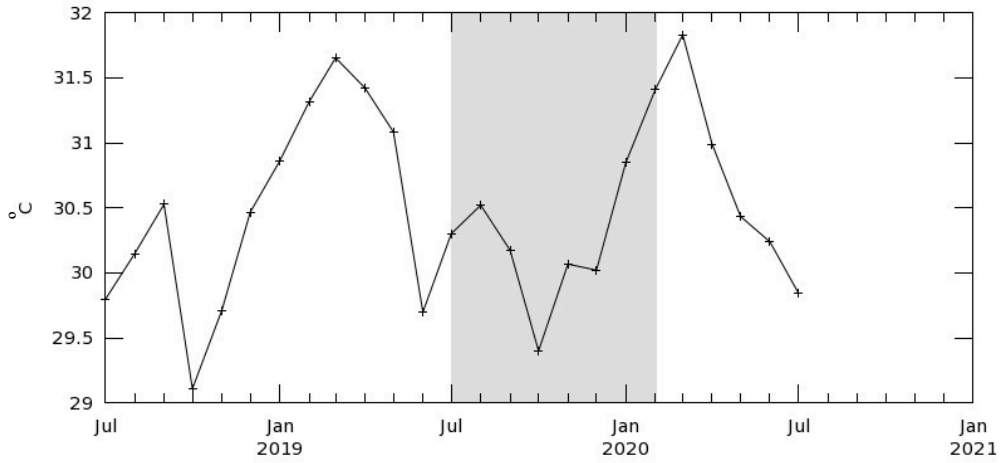

Figure 3. Area-averaged daytime sea surface temperatures (ºC) from the northern tip of Golfo Dulce from July 2018 to July 2020. The timeframe of the experiment is shaded in gray. Data from the NASA GIOVANNI satellite.

Study organisms: Although no survey has been conducted describing the species of cryptic fauna associated with *Pocillopora* in Golfo Dulce apart from coral borers

(Fonseca & Cortés 1998), those expected to be present can be predicted from surveys conducted in other reefs in the ETP including Bahía Culebra, Costa Rica (Alvarado and Vargas-Castillo 2012, Salas-Moya et al. in prep.), Uva Island in Panama (Abele & Patton 1976, Glynn 1980, Gotelli and Abele 1983, Gotelli et al. 1985), and the Pacific coast of Mexico (Hernández et al. 2009, 2013). While the ichthyofauna of Golfo Dulce has some similarities with that of the rest of the Pacific coast of Costa Rica, it is statistically different in terms the species composition (Alvarado et al. 2014).

Out-planting of nursery-grown *Pocillopora* colonies and experimental design: A total of 30 *Pocillopora* colonies were transplanted at the restoration site in Punta Bejuco in July 2019. The colonies were anchored with nylon zip-ties to steel nails, driven into the calcium carbonate substrate in a 6 by 5 colony rectangular arrangement with a distance of 30cm from neighboring colonies. The rectangular transect extends 15cm from the centroids of the fringing nails to measure 1.5 by 1.8 m in total (Fig. 4). In order to understand how the presence of the transplanted coral affects the fish and benthic invertebrate communities and the sea floor cover, a control transect, devoid of coral transplants was designated and monitored using the same methods as the transplantation site. The control transect is located at Punta Bejuco at the same depth as the transplant area in a relatively flat region with a (visually) similar substrate composition. This transect was prepared in the same layout of 30 steel nails as the experimental transplanted transect, but contains no *Pocillopora* transplants (Fig. 4).

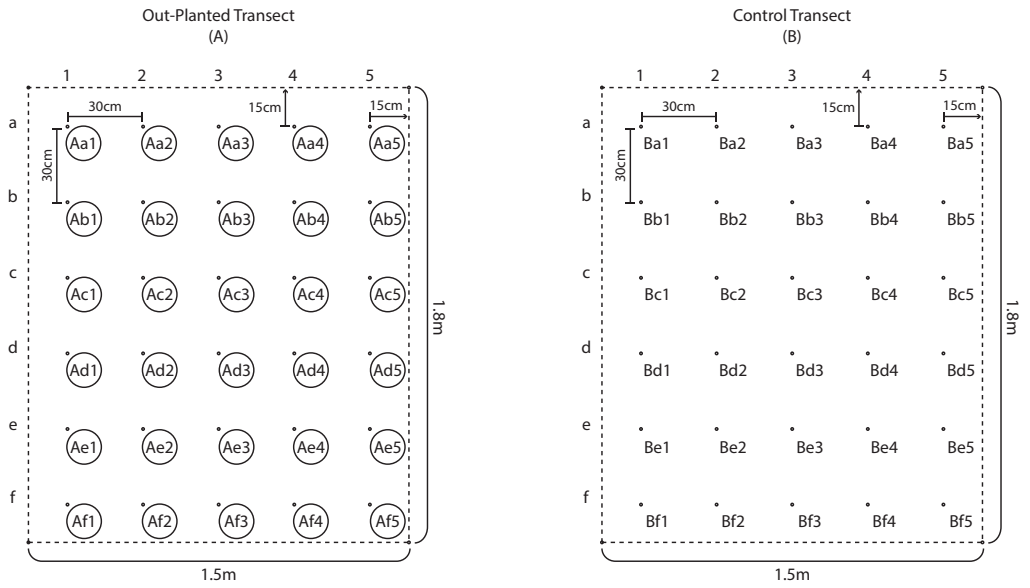

Figure 4. The arrangement of experimental colonies and nails within transects on the sea floor. The black dots represent nails used as attachment points for the colonies or as markers of transect area and quadrat centers. The white circles represent *Pocillopora* colonies. The dotted line represents the edge of the experimental and control transects. Each colony or nail position is given a procedural name so that it can be easily located.

Monitoring of cryptic fauna: In the transplanted transect, the cryptofaunal symbionts in each colony were identified and counted by visual census every month between July 2019 and February 2020. Twelve months of data collection were planned, but due to the COVID-19 global pandemic, field trips were terminated at eight months. The observer only identified and recorded the organisms occupying the space between the branches or on the surface of each colony as cryptofauna. Most methods used to investigate the cryptofauna living between the branches of corals require the corals to be removed from the study site and destroyed (Abele & Patton 1976, Enochs & Hockensmith 2008, Stella et al. 2010, Alvarado & Vargas-Castillo 2012, Enochs & Manzello 2012a,b). *In situ* visual identification provides a low-cost, non-destructive method for the quantification of the cryptic community over time, although it is not as precise as destructive collection in the identification of very small and burrowing cryptic species (Alzate et al. 2014; Caldwell et al. 2016) that are not the focus of this study.

Monitoring of ichthyofauna communities in transplanted and control transects: To identify and count the reef-associated fish in either transect, each month a diver observed ichthyofauna from a fixed position approximately 2m away from the deepest border of the out-planted transect and the control transect. The observer identified and counted fish within an imaginary rectangular prism extending the length and width of each transect and 3 m above the sea floor (Fig. 5). The icthyofauna community associated with the *Pocillopora* outplants and control transect was summarized by three measurements: Shannon diversity (H'), total abundance of fish, and the specific abundance of each species.

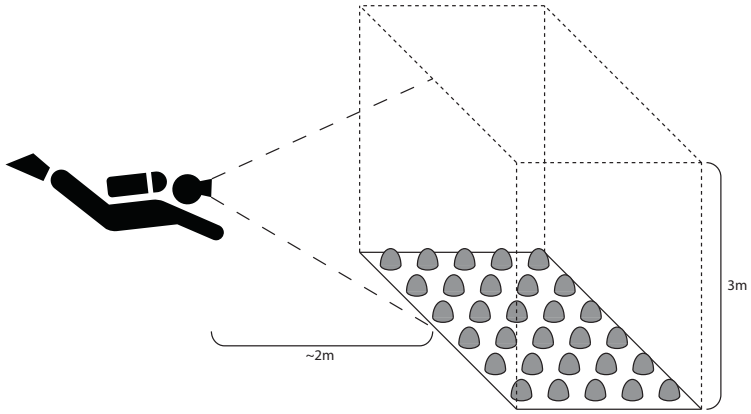

Figure 5. Method for counting coral-associated fish at the experimental and control sites.

Data analysis:

Cryptofaunal community: The diversity and abundance of cryptic organisms is described in terms of operational taxonomic units (OTUs) instead of species according to Enochs & Manzello (2012b), to prevent overestimation. The cryptic faunal community associated with the *Pocillopora* outplants is summarized in terms of the number of OTUs, total abundance of cryptic organisms, and the specific abundance of each OTU in each colony and within the experimental patch as a whole. The diversity of the cryptofauna was defined as the number of unique OTUs present in each colony.

Linear regression was carried out using the statistical package R (R Core Team, 2013) to estimate the change in the abundance and diversity over the 8 months of data

collection. To explore changes in the cryptofaunal community living between the branches of the transplanted *Pocillopora* colonies, the similarity of community composition in terms of the presence and abundance of cryptofauna OTUs between and across months and seasons (dry, transition and rainy) was quantified with cluster analysis performed in PRIMER 7. First, the average abundance of each cryptic species each month was square root transformed in order to enhance the impact of less common OTUs and to diminish that of the most common OTUs. The transformed data were then used to build a Bray-Curtis similarity matrix (Clarke & Gorley 2015). This similarity matrix was used to represent the similarity of the cryptic community each month in two (2) dimensions using non-metric multidimensional scaling (nMDS) (Clarke & Gorley 2015) and to build a dendrogram showing the similarity of the cryptic community of the transplanted colonies between sample months. Additionally, the data were used to generate a heat plot that displays the contribution of each OTU to the cryptic community each month. The data were labeled by their seasonal groupings as described earlier: "Wet" season includes September, October, November & December, "Transition" season includes July & August, and "Dry" season includes January and February.

To test for significant difference in the cryptic community between seasonal groupings of months in terms of the average abundance of OTUs, an analysis of similarity (ANOSIM) was conducted using the Bray-Curtis similarity matrix. To determine which OTUs contributed most to any dissimilarity in the cryptofaunal community between groupings, similarity percentage analysis (SIMPER) was conducted using the Bray-Curtis similarity matrix in PRIMER 7.

Ichthyofaunal community: Student's t-tests were performed in R to detect differences between the experimental and control transects in the abundance (number of individuals per patch) and Shannon diversity (H') of the ichthyofauna over the eight months of data collection. nMDS was performed in PRIMER 7 to explore differences in the reef fish community between the experimental and control patches. The abundances of fish species each month were square root transformed in order to enhance the impact of less common species and to diminish that of the most common species. The transformed data were then used to build a Bray-Curtis similarity matrix (Clarke & Gorley 2015). This

similarity matrix was used to represent the similarity of the fish community each month in two (2) dimensions using non-metric multidimensional scaling (nMDS) (Clarke & Gorley 2015). Differences in the reef fish community between experimental and control patches were explored with ANOSIM and SIMPER in PRIMER 7.

RESULTS

Description of the cryptofaunal community: Over the eight months of the study, a total of 17 OTUs of cryptofauna were identified and counted (Table 1). These OTUs were split into four categories based on their taxonomy and ecological role in the reef ecosystem: (1) decapod crustaceans, (2) cryptic fish that permanently occupied the spaces between the coral branches, (3) reef fish that are usually encountered in the water column around corals, and (4) encrusting animals attached to the coral transplants. The decapod crustaceans included *Trapezia* spp., *Alpheus lottini*, *Harpiliopsis depressa*, *Palaemonella holmesi*, *Palaemon ritteri*, the family Paguridae and the superfamily Majoidea. The cryptic fish all fell within the order Blenniiformes and were categorized as such in Table 1. Species that could be identified in this group included the species *Axoclinus lucilae*, *Acanthemblemaria exilispinus*, *Elacatinus punticulatus*, *Plagiotremus azaleus* and unidentified species within the family Tripterygiidae. Reef fish included *Abudefduf troschelli*, *Scarus* spp. and *Stegastes acapulcoensis* and fish in the family Chaetodontidae. The encrusting metazoan group included polychaetes within the family Sabellidae, ascidians, gastropods within the family Vermetidae and bivalves within the genus *Spondylus*.

Table 1. Counts and percentages of cryptofauna OTUs identified within the transplanted corals each month following transplantation at Punta Bejuco, Golfo Dulce. Symbols in parentheses are used in Figure 8. Asterisks (*) indicate OTUs that are obligate symbionts of *Pocillopora* spp. OTUs are arranged by order of first observation and percent contribution to the cryptofaunal community each month.

Group	O T U (symbol)	July		August		September		October		November		December		January		February	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Decapod	***Trapezia* spp.*** (tra)	168	54.7%	117	50.9%	153	45.0%	128	30.8%	153	49.7%	181	51.9%	252	37.9%	250	38.9%
Decapod	***Harpiliopsis depressa**** (hde)	103	33.6%	73	31.7%	83	24.4%	183	44.0%	79	25.6%	50	14.3%	238	35.8%	157	24.4%
Decapod	***Alpheus lottini **** (alo)	24	7.8%	38	16.5%	90	26.5%	89	21.4%	46	14.9%	102	29.2%	119	17.9%	91	14.2%
Encruster	***Spondylus* sp.** (spo)	8	2.6%														
Decapod	***Palaemonella holmesi*** (pho)	4	1.3%													46	7.2%
Decapod	**Majoidea** (maj)			2	0.9%												
Cryptic fish	**Blenniiformes** (blenny)					4	1.2%	4	1.0%					18	2.7%	2	0.3%
Decapod	**Paguridae** (pagu)					4	1.2%	8	1.9%	10	3.2%	4	1.1%	10	1.5%	8	1.2%
Encruster	**Vermetidae** (verm)					2	0.6%	2	0.5%	10	3.2%	4	1.1%			2	0.3%
Encruster	**Ascidea** (asci)							2	0.5%	2	0.6%	6	1.7%				
Encruster	**Sabellidae** (sab)									8	2.6%	2	0.6%				
Reef fish	***Scarus* spp.** (scar)													24	3.6%	27	4.2%
Reef fish	***Stegastes acapulcoensis*** (sac)													4	0.6%	8	1.2%
Decapod	***Palemon ritteri*** (pri)															42	6.5%
Reef fish	**Chaetodontidae** (chaet)															8	1.2%
Reef fish	***Abudefduf troschelli*** (atr)															2	0.3%
TOTAL Abundance		307	100%	230	100%	340	100%	416	100%	308	100%	349	100%	665	100%	643	100%
	Total OTUs	5		4		6		7		7		7		7		12	

Patterns in the community of cryptofauna in *Pocillopora* colonies following transplantation

The dominant cryptofauna over the course of the experiment were *Pocillopora* symbiont decapods in the OTUs *Trapezia* spp., *Harpiliopsis depressa* and *Alpheus lottini.* They comprised on average 81% (43%, 30% and 18% respectively) of the fauna identified within the 30 colonies over the 8 months of observations (Table 1). The rest of the cryptofauna identified within the colonies were cryptic fish, transient fish and encrusting metazoans accounting for 18.93% of the associated fauna found between the branches of the *Pocillopora* transplants over the course of the study.

Different groups of cryptofauna were observed within the colonies in sequential order (Table 1). The first group is composed of decapods that were present in the colonies prior to transplantation in July 2019. The second group to appear was the cryptic fish,

first appearing in September 2019, disappearing November and December and reappearing in January and February 2020. The next group to appear was the sessile encrusting organisms, first appearing in July 2019 but then disappearing in August. The final group to appear was the reef fish in January 2020.

Abundance: In July 2019, the day following the transplantation of the colonies, an average of 10 individual cryptic organisms were observed per colony (ind colony^{-1}). In February 2020, an average of 21 ind colony^{-1} were observed. Based on linear regression, the abundance of cryptofauna living within the transplanted coral colonies increased significantly with time (R^2 = 0.29, $p < 0.001$) over the eight months following transplantation (Fig. 6).

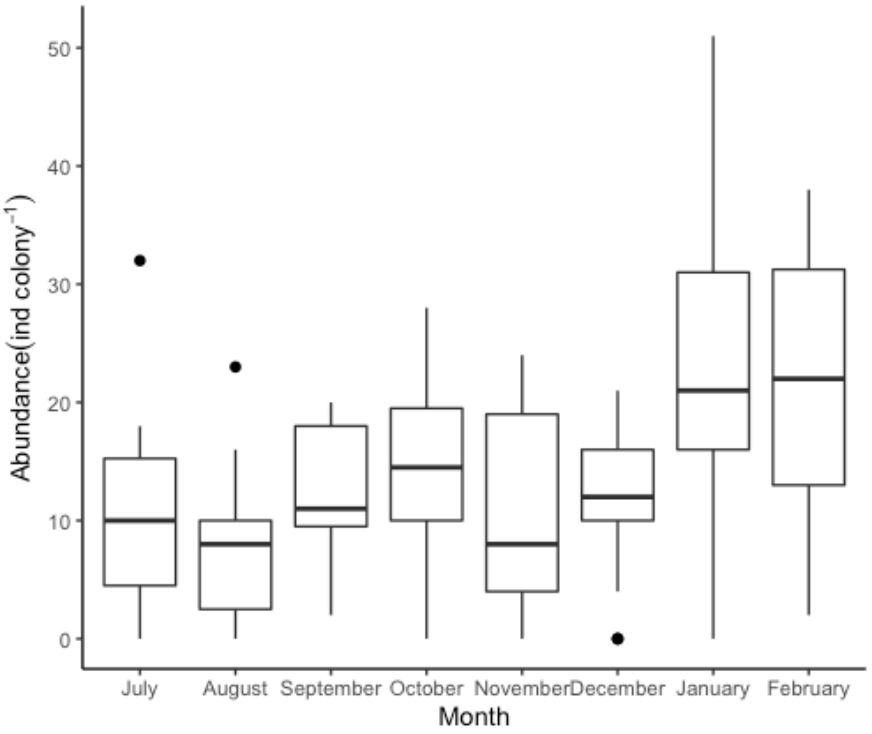

Figure 6. Number of individuals of cryptofauna living within the branches of each *Pocillopora* colony (ind colony^{-1}) over the eight months of data collection.

Diversity: The diversity of cryptofauna within the transplanted coral colonies increased significantly with time (Linear regression: $R^2 = 0.17$, $p < 0.001$) in the eight months following the transplantation (Fig. 7). In the entire experimental patch, five (5) OTUs were present at the initial observation in July 2019 which increased to 12 OTUs by the end of the eight months of data collection in February 2020 (Table 2).

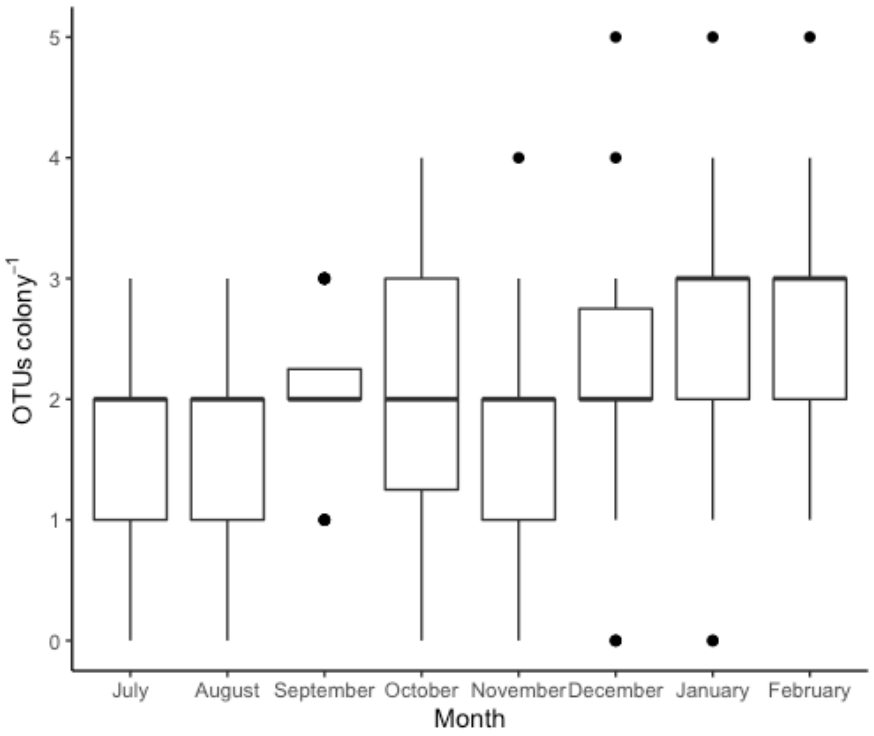

Figure 7. Diversity of cryptofauna living between the branches of the experimental *Pocillopora* colonies in the eight months following transplantation, in terms of the number of OTUs identified in each colony each month

Monthly change in cryptofaunal community composition: Non-metric multidimensional scaling (nMDS) (fig. 8) and the corresponding cluster analysis

dendrogram (Fig. 9) showed 3 distinct groupings of average monthly cryptofauna community compositions (>80% similarity within groups) corresponding to seasonal groupings: September, October, November & December which corresponds with the "Wet" season, July and August which corresponds to the "Transition" season, and January and February which corresponds with the "Dry" season (note that the months March, April, May and June were not sampled). Analysis of similarity (ANOSIM) was used to detect differences between these groups. The groups were shown to be well separated (R statistic) but not significantly different (p statistic) in composition (global R = 0.988, global p = 0.05; Dry vs. Trans.: R = 1, p = 0.33; Dry vs. Wet: R = 1, p = 0.07; Trans. vs. Wet: R = 0.96, p = 0.07). Results of similarity percentage analysis (SIMPER) show that the three groups differ more from each other than within each group (Table 2).

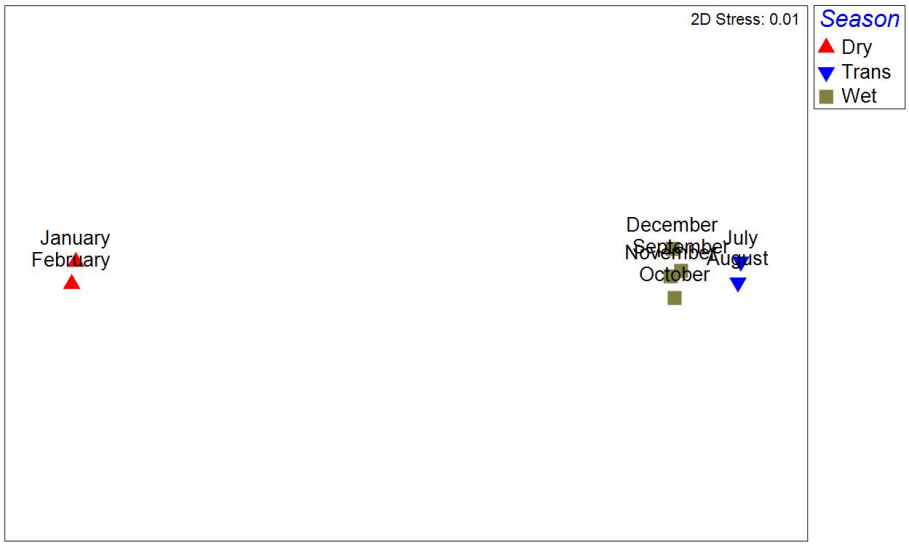

Figure 8. Non-metric multidimensional scaling of the composition of cryptofauna within the transplanted *Pocillopora* colonies in the experimental patch, Punta Bejuco, Golfo Dulce for each month based on Bray-Curtis similarity matrices built on square-root transformed monthly average abundances of cryptofauna OTUs and by the "season" as a factor.

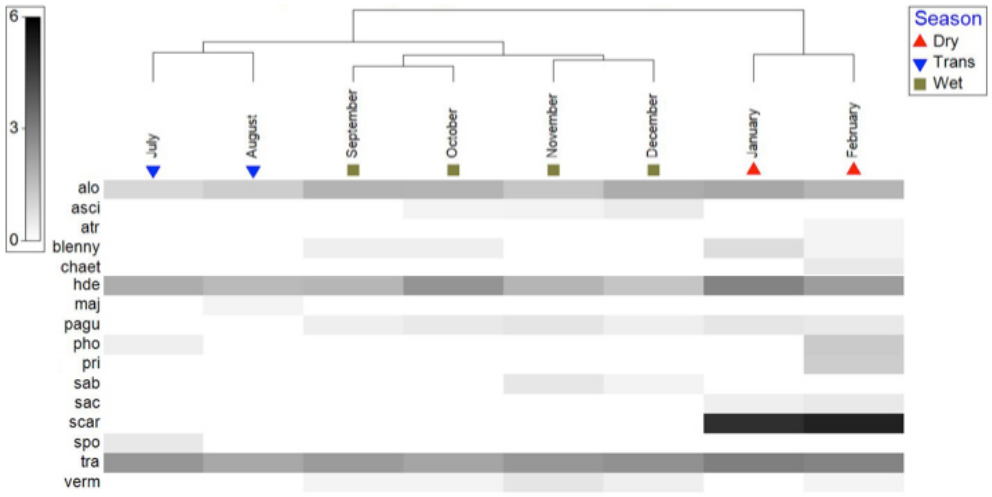

Figure 9. Average union dendrogram showing the similarity of the cryptofaunal community of the *Pocillopora* transplants between months in the experimental patch, Punta Bejuco, Golfo Dulce. Months are arranged chronologically from left to right and grouped by the factor season. Below the dendrogram is a heatplot showing the square-root transformed abundances (ind colony $^{-1}$) of cryptofauna each month. OTU symbols are assigned in Table 1.

Table 2. SIMPER table showing the similarity/dissimilarity within and between seasons and the percentage difference the most significant OTUs contribute.

Season	% Similarity/Dissimilarity	OTU	% Contribution
Dry	82.3% Similar	*Scarus* spp.	37.9%
		Trapezia spp.	22.2%
		Harpilliopsis depressa	17.6%
Transition	85.3% Similar	*Trapezia* spp.	45.8%
		Harpilliopsis depressa	36.2%
Wet	84.3% Similar	*Trapezia* spp.	35.4%
		Harpilliopsis depressa	25.1%
		Alpheus lottini	24.9%
Dry & Transition	46.1% Dissimilar	*Scarus* spp.	38.7%
		Trapezia spp.	15.7%
		Harpilliopsis depressa	10.1%
		Palaemonella holmesi	4.7%
		Palemon ritteri	4.4%
Dry & Wet	44.2% Dissimilar	*Scarus* spp.	38.1%
		Trapezia spp.	12.2%
		Alpheus lottini	10.4%
		Harpilliopsis depressa	7.4%
		Vermetidae	4.7%
Transition & Wet	23.6% Dissimilar	*Alpheus lottini*	15.2%
		Paguridae	14.6%

Vermetidae	11.8%
Harpilliopsis depressa	11.6%
Spondylus sp.	8.7%
Ascidea	7.6%
Trapezia spp.	7.1%

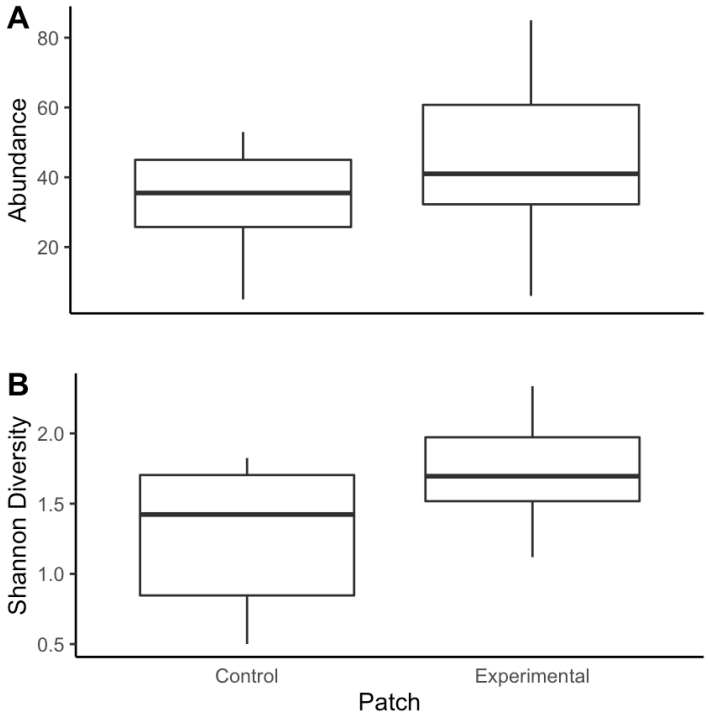

Figure 10. Average abundance (A) and average Shannon diversity (B) of fish in experimental and control patches over the course of the experiment.

Table 3. Species observed and average monthly observations in the experimental and control patches following transplantion. The gray shading highlights which species were observed more often in each patch.

		Average observations per month	
Species	**Ecological role**	Experimental	Control
Stegastes acapulcoensis	Detritivore	10.6	14.0
Stegastes flavilatus	Detritivore	1.5	0.1

Anisotremus taeniatus	Invertebrivore	0.0	0.1
Arothron hispidus	Invertebrivore	0.3	0.1
Bodianus diplotaenia	Invertebrivore	0.0	0.8
Canthigaster punctatissima	Invertebrivore	1.9	0.8
Gnathanodon speciosus	Invertebrivore	1.5	0.0
Haemulon steindachneri	Invertebrivore	1.8	0.0
Halichoeres dispilus	Invertebrivore	2.3	6.0
Halichoeres nicholsi	Invertebrivore	0.1	0.0
Johnrandallia nigrirostris	Invertebrivore	0.1	0.0
Pomacanthus zonipectus	Invertebrivore	0.1	0.1
Sphoeroides lobatus	Invertebrivore	0.8	0.8
Thalassoma lucasanum	Invertebrivore	2.9	1.8
Kyphosus elegans	Macroalgae feeder	0.1	0.0
Scarus compressus	Macroalgae feeder	0.3	0.8
Scarus ghobban	Macroalgae feeder	6.6	3.0
Chaetodon humeralis	Omnivore	0.1	0.0
Kyphosus ocyurus	Omnivore	0.8	0.0
Cephalopholis panamensis	Piscivore	1.9	0.3
Haemulon maculicauda	Piscivore	3.4	2.9
Haemulon sexfasciatum	Piscivore	0.1	0.0
Lutjanus argentiventris	Piscivore	3.9	0.4
Lutjanus novemfasciatus	Piscivore	0.0	0.5
Abudefduf troschelii	Planktivore	4.0	0.9
	Total	44.9	33.1

Changes in the reef fish community following transplantation: Although there are trends toward greater abundance and diversity of reef fish in the experimental compared to the control patch, these trends were not statistically significant: Abundance (Student's t-test: df = 11.72, p = 0.28) (Fig. 10A) and Shannon diversity (Student's t-test: df = 13.03, p = 0.061) (Fig. 10B). 22 and 17 species were observed in the experimental and control patches respectively. The average numbers of individual fish observed each month were 44.9 and 33.1 in the experimental and control patches, respectively (Table 3). The species with the greatest number of observations was *Stegastes acapulcoensis*. The ichthyofaunal community did not differ significantly between the control and experimental patches based on the presence and abundance of fish species for the eight months of collected data (ANOSIM R = 0.143; p = 0.074) (Fig. 11).

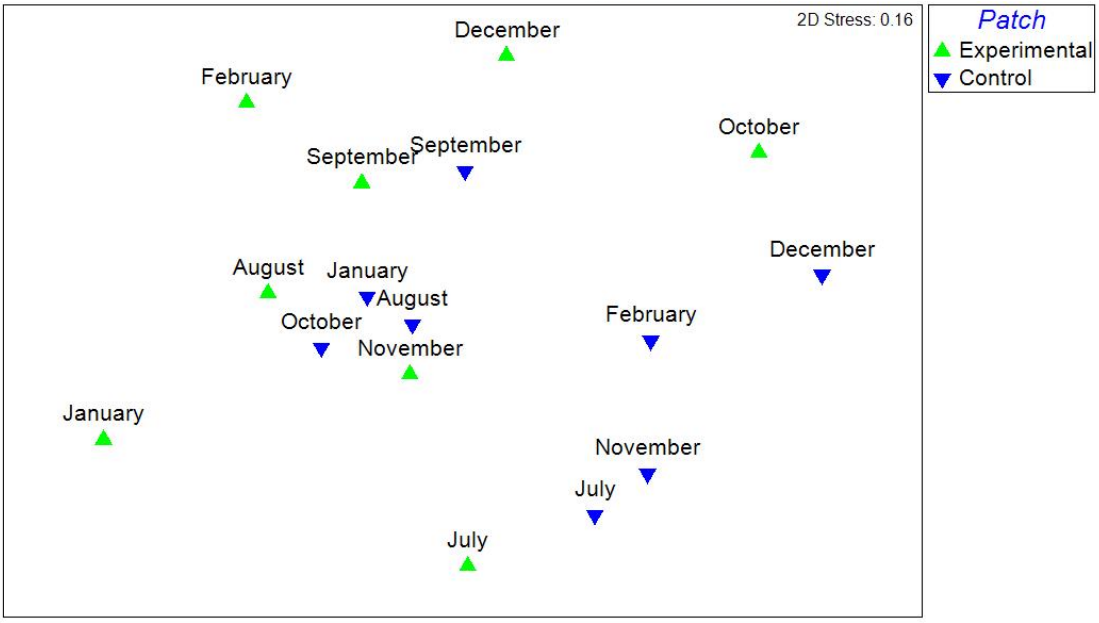

Figure 11. Non-metric multidimensional scaling of the composition of the fish community within the experimental and control patches at Punta Bejuco, Golfo Dulce each month based on Bray-Curtis similarity matrices built on square-root transformed monthly averages of fish species abundances. The experimental and control patches are indicated with differing symbols and point colors.

Table 4. SIMPER showing the percent similarity in fish communities within and between experimental and control patches at Punta Bejuco, Golfo Dulce and the contribution of species to that similarity.

Patch	% Similarity/Dissimilarity	Species	% Contribution
Experimental	42.0% Similar	*Stegastes acapulcoensis*	33.84
		Scarus spp.	17.06
		Abudefduf troschelii	10.71
		Halichoeres dispilus	9.36
		Lutjanus argentiventris	7.69
Control	50.0% Similar	*Stegastes acapulcoensis*	56.34
		Halichoeres dispilus	25.5
Experimental & Control	57.4% Dissimilar	*Scarus* spp.	12.16
		Abudefduf troschelii	9.43
		Haemulon maculicauda	8.88
		Halichoeres dispilus	8.58
		Lutjanus argentiventris	7.99
		Stegastes acapulcoensis	7.77

DISCUSSION

Over the course of the eight months following transplantation to the restoration site, the cryptofaunal community was dominated by obligate decapod symbionts: *Trapezia* sp., *Harpiliopsis depressa* and *Alpheus lottini*. This is consistent with the hypothesis that these species only live within *Pocillopora* (Abele & Patton 1976; Castro 1988; Stella et al. 2011) and maintain a constant presence independent of season (Gotelli et al. 1985). Likewise, the *Pocillopora* colonies require the symbionts to survive and grow (Stella et al. 2011, Glynn 2013). The abundance of cryptofauna more than doubled over the observational period, from an average of 10 individuals in each of the 30 transplanted corals on the day after transplantation to 21 individuals eight months later ($p<0.05$). The diversity of the experimental patch also increased significantly, with 5 OTUs in the first month and 12 OTUs the final month.

The cryptofaunal community observed at the study site reflects the known ecology of reef fauna reported in the literature. Decapod crustaceans are the most abundant and diverse cryptic symbionts (Reaka-Kudla et al. 1997, Stella et al 2011, Glynn 2013). The brachyuran crabs of the genus *Trapezia* and snapping shrimp of the genus *Alpheus* are common decapod symbionts associated with the highly branching coral *Pocillopora*. The decapods defend their host coral from coralivorous invertebrates like the sea star *Acanthaster plancii* (Pratchett 2001, Glynn 2013) or the snail *Drupella* (McKeon & Moore 2014, Samsuri et al. 2018) by snapping at intruders. Symbiotic decapods reduce coral mortality due to sedimentation (Stewart et al. 2006, Stier et al. 2012) and disease (Pollock, Katz & Willis 2013) by actively "kicking" and "shoveling" unwanted particles from *Pocillopora* colonies, thus reducing stress on the coral (Stewart et al. 2006). The presence of *Trapezia* crabs induces the *Pocillopora* host to produce nutrient rich mucus with fat globules in order to encourage the mutualism between host coral and crab (Stimson 1990).

The change in diversity of the fauna living within *Pocillopora* colonies over time appeared to represent different recruitment periods of the ecological groups (Table 2). The initial recruits were the obligate symbiont decapods that were recruited to the colonies while they grew in the coral nurseries in Nicuesa (personal observation) and remained present following the transplantation of colonies at Punta Bejuco. The next

recruits, first observed in September, three months post-transplantation, were blennies, cryptic fish, followed by encrusting animals. Others have observed that as branching coral colonies grow, the tissue on the bases of the branches begins to die and sessile encrusting and boring animals are able to colonize the exposed carbonate skeleton (Abele & Patton 1976, Enochs & Manzello 2012a). The final group of animals observed to be recruited to the transplanted colonies were larger reef fish.

Although not significantly different, the results of the nMDS, cluster analysis and SIMPER (Fig. 6) suggest that the cryptic communities were similar within groupings of months that roughly correspond to commonly held ranges of the three seasons in Costa Rica, as defined by the amount of precipitation of that period of the year (Table 1).

The increase in abundance and diversity of cryptofauna over time could be explained by multiple processes. The first regards the growth of the coral. As corals grow, more space is available between their branches to harbor symbionts, thus reducing competition for space and allowing fauna abundance to increase over time with the size of the coral colonies (Abele & Patton 1976). However, intense and complex inter- and intraspecific aggressive interactions in the common *Pocillopora* symbionts *Trapezia* and *Alpheus* keep the diversity of the obligate symbionts constant and prevent other organisms from being recruited (Huber 1987; Castro 1996; Stier et al. 2012). This could explain why over the course of the experiment, obligate symbionts in just three OTUs comprised 81% of the total number of individuals that occupied the experimental coral transplants.

Other explanations could, as implied by the seasonal groupings of monthly cryptofaunal community compositions, be the result of seasonal changes in environment. In the ETP, the recruitment of associated fauna is greater in the warmer months due to increased metabolic rate and reproduction of invertebrates (Rodríguez-Troncoso et al. 2018). The environmental factor that most greatly distinguishes the seasons in Golfo Dulce is precipitation (Cortés 1990, Quesada & Cortés 2006) leading to greater sedimentation in the gulf during the rainy season. This sedimentation has physiological consequences for the coral such mucus production and necrosis that could have effects on the associated fauna (Fabricius 2005).

Ecological succession of species through facilitation could be another explanation for the increase in diversity and the sequential appearance of different groupings of fauna following the transplantation of the *Pocillopora* colonies. Although this study examines succession only insofar as to describe the order of appearance of different of species, the mechanisms behind these patterns can be speculated. The presence of cryptofauna may attract larger species looking to feed on small invertebrates living within the coral who themselves bring symbionts and nutrients to the growing ecosystem.

As expected, a greater diversity and abundance of fish were observed in the experimental patch containing colonies rather than the control patch without colonies although the differences were not statistically significant. Fish are attracted to structural complexity because it provides numerous microhabitats that coincide with greater diversity of prey animals and shelter whether it is a live reef (Bel & Galzin 1984) or an artificial reef (Russel 1975, Santos et al. 2011, Becker et al. 2017). Herbivorous fish control the population of leafy and fleshy algae that can impede the settlement and growth of corals (Hixon & Bronstoff 1996, Burkpile & Hay 2010, Humphries et al. 2014). There may be species-specific effects of different herbivores on different types of algae, meaning that fish diversity is important for the health of reefs (Burkpile & Hay 2010)

The cryptofaunal community in this study behaved differently in some respects when compared to descriptions in the literature. For example, intraspecific competition normally limits species in the genera *Alpheus* and *Trapezia* to two individuals of the same species per colony, a male and female reproductive pair (Huber 1987; Castro 1996; Stier et al. 2012). In this study, we observed an average of 5.8 *Trapezia* individuals per colony, and 2.5 *Alpheus* individuals per colony. This could be explained by the limited population of *Pocillopora* in Golfo Dulce resulting in fewer places for competing decapod symbionts to move (Castro 1978).

CONCLUSIONS

Coral reefs are in decline globally (Sandin et al. 2008, Hughes et al. 2017) and in Costa Rica specifically, due to many factors including climate change, pollution, inadequate planning of coastal development, poorly managed fisheries, and eutrophication (Cortés & Jiménez 2003). As the corals die off, so do the fauna that depend on the reefs to survive (Enochs & Hockensmith 2008, Glynn 2011). In turn, these reef-associated fauna are critical to the health of the corals and without them the process of recuperation is not possible (Bel & Galzin 1984; Hancock, Lewis & Conklin 2017). This results in the decline of ecosystems throughout the ocean and has economic impact on communities that depend on them for their livelihood (Costanza et al. 2014). It has been observed that reef ecosystems destroyed by anthropogenic disturbance do not tend to recuperate without human intervention under current environmental conditions (Rinkevich 2005). Coral reef restoration aims to rebuild coral reef communities through active reconstruction of the physical structure (Horoszowski-Fridman et al. 2015, Hancock, Lewis & Conklin 2017). The ultimate goal is to encourage the recruitment of coral-associated fauna (Yap 2009, Ladd et al. 2019) that, in turn, encourage the recuperation of biodiversity and ecological processes that are required for the survival of the reef (Bel & Galzin 1984; Hancock, Lewis & Conklin 2017).

Over the course of eight months following transplantation of 30 *Pocillopora* colonies in Golfo Dulce, the abundance and diversity of cryptofauna increased significantly. Obligate symbionts were the first recruits to the transplanted corals and the dominant cryptofauna. The community composition also varied somewhat by distinct temporal groups that correspond with seasons in the Golfo Dulce. The abundance and diversity of fish were higher in the experimental patch that was transplanted with coral, than in the control patch, although the results were not statistically significant.

The data collected for this project was used to add to our understanding of the interrelationships between associated fauna and coral, the patterns of biodiversity, and ecological succession in *Pocillopora* transplants in the restoration project in Golfo Dulce, South Pacific, Costa Rica. While not extensively generalizable, the results may be useful for helping to plan future restoration projects and to guide expectations regarding the magnitude of the number and variety of cryptic organisms in the immediate aftermath of coral transplantation and the expected timing of the recruitment of associated fauna.

Multiyear studies that include extensive environmental monitoring are required to tease out the relative contribution of environment, interspecies interactions, and the other multiple contributions to the complex reef colony. This type of understanding of coral reef ecosystems is one of the most critical ways to combat the ecological crisis of coral decline (Bellwood et al. 2004).

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Chapter II:

Succession of the sessile benthic community at a coral reef restoration site, Golfo Dulce, South Pacific Costa Rica

Master's thesis of:

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ABSTRACT

Ecosystem restoration is essentially a facilitation of ecological succession. When a coral reef experiences a disturbance, the community of sessile benthic organisms can follow a successional trajectory that favors the dominance of coral or a change of state to an ecosystem dominated by aggressive algae that prevent the growth and settlement of coral depending on environmental and ecological conditions. The objectives of this study were to monitor and measure the growth and health of the transplanted corals, and to describe the effect of the transplantation of *Pocillopora* colonies on the succession of the sessile benthic community. Experimental and control patches were established at the restoration site in Golfo Dulce. Thirty *Pocillopora* colonies, grown from fragments of native corals were attached to nails on the substrate; the control patch contained nails in the same arrangement, but without colonies. Every month for 8 months, each coral or nail was photographed from above using a camera in a PVC frame for reference. Areas of coral and other sessile benthic organisms were extracted from the images using PhotoQuad. The growth rate of the area of the colonies (cm^2) was calculated from the data extracted from the photographs with linear regression. Non-metric multidimensional scaling was used to describe changes in the sessile benthic community of the reef and understand the impact of the presence of the coral transplants on succession by comparing the initial and final community compositions of the experimental and control patches. The coral transplants experienced two bleaching events, the first in August-September 2019 and the second in January-February 2020. By the end of the 8 months of the experiment, 5 of the initial 30 colonies had died and 20 were bleached, leaving 5 healthy colonies. The colonies grew significantly in area following transplantation; 67% of their initial area after 8 months. The sessile benthic community changed in the control patch and remained constant in the control. In the experimental patch, the areas of *Pocillopora*, coralline crustose algae (CCA), and cyanobacteria increased while the area of algal turf decreased. The first bleaching event could have resulted from rainier conditions and sedimentation or transplantation stress. The second could have resulted from higher sea surface temperatures, exacerbated by the shallow depth of the transplants. The increase in coral coverage and CCA and decrease in algal turf in the experimental patch could be due to herbivores attracted to the structural complexity of the transplants. The increase in cyanobacteria in the experimental patch could be the result of higher temperatures and may have been a factor in the death of colonies. The transplantation of *Pocillopora* colonies in Golfo Dulce may have changed the successional trajectory to favor the growth and survival of coral in the experimental patch. This trajectory should be monitored over longer temporal and larger spatial scales to observe whether the trends continue. This information is useful in informing future restoration efforts on what to expect in the early succession of the sessile benthic community around coral transplants.

Key Words: Phase shift, eastern tropical Pacific, algae, coral bleaching

INTRODUCTION

Coral reefs are in decline globally due to many factors including climate change, pollution, inadequately planned coastal development, mismanagement of fisheries, and eutrophication (Sandin et al. 2008, Van Oppen et al. 2017). In addition to the ecological impact, this decline has economic impact on societies that depend on coral reefs for their livelihood (Costanza et al. 2014). Under current environmental conditions, reef ecosystems destroyed by anthropogenic disturbance do not tend to recuperate unless the disturbance is reduced and there is human intervention (Rinkevich 2005). Coral reef restoration is one form of human mediation that aims to rebuild coral reef communities through active propagation of coral in order to promote the recovery of the ecosystems' natural complement of diversity and ecological functionality (Horoszowski-Fridman et al. 2015, Hancock, Lewis & Conklin 2017). The most common method of coral reef restoration is through coral gardening and transplantation (Bayraktarov et al. 2019).

Coral restoration is essentially a facilitation of natural successional processes through the reintroduction and management of key species (Young 2001, Walker et al. 2007). Ecological succession is the process by which an ecosystem undergoes a predictable sequence of the replacement of communities that reach a stable climax and remains reasonably unchanged over time until the next disturbance (Odum 1964). The early succession of a reef is critical to the establishment, survival and resilience of coral reef communities (Doropolous et al. 2016). For example, algal turf impedes the settlement of coral larvae and the growth of colonies (Smith et al. 2010, O'Brien & Schiebling 2018). Conversely, coralline crustose algae promote the settlement and growth of corals (Doropolous et al. 2016).

Coral growth rate is one of the most widely used assessments of coral reef health and restoration success (Edmunds & Putnam 2020). The growth of corals can be affected by environmental factors including temperature (Jiménez & Cortés 2003, Manzello 2010), seawater pH (Manzello 2010, Guo et al. 2020), nutrients (Toh et al. 2014), light (Wellington 1982), depth, and biological factors such as the presence of certain fish,

invertebrates (Glynn & Enochs 2010) and algae (Lirman 2001). Scientists collect coral growth information to assess the environmental conditions and locations where corals can survive and thrive (Edmunds & Putnam 2020).

The succession of coral reefs occurs at short time scales in the range of months and geological time scales that occur over centuries, millennia or even millions of years (Grigg 1983, Karlson 1999). At short time scales, succession is localized on small patches of substrate and is dominated by fast-growing organisms such as algae competing for light, space, and other resources. This short time scale succession is the most relevant to processes of coral recruitment, competition and growth (Doropolous et al. 2016). Coral reef succession at geological time scales is characterized by turnovers of species of hermatypic corals and the physical structure of the reef as long-term changes in the ecology, environment, and frequency of disturbance favor the growth of certain species and forms over others (Grigg 1983). For example, fast-growing branching corals such as pocilloporids and acroporids tend to be early colonizers of a new reef that are sensitive to environmental changes and are replaced by slower growing, more tolerant, massive species that build on the physical and ecological foundations of the branching species, eventually replacing them as the dominant growth forms (Grigg 1983).

Coral reefs are ecosystems characterized by constant disturbance, including anthropogenic and natural factors. This constant disturbance contributes to the high diversity of life in coral reefs (Grigg 1983) and also their vulnerability to decline. Disturbances such as high sediment loads, elevated temperatures from El Niño Southern Oscillation (ENSO) events stress corals and cause them to lose their zooxanthellae symbionts and to "bleach". If a disturbance is strong and persistent, a coral reef ecosystem can settle into alternate stable states dominated by different organisms depending on environmental and ecological factors (Dudgeon et al. 2010, Fung et al. 2011). Thus, as succession follows a disturbance, its trajectory can be affected by these environmental and ecological factors. For example, under conditions of constant disturbance, climate change and other anthropogenic factors tend to push recovering ecosystems to favor the proliferation of macroalgae and turfs instead of reef-forming

corals. Algae-dominated marine ecosystems often do not naturally revert to coral-dominated ecosystems without intervention (Smith et al. 2010). This is because reef-forming corals tend to be out-competed by algal turfs that often flourish in ecosystems with high water nutrient concentrations and fewer herbivores; a condition that can occur in ecosystems degraded by eutrophication and poorly regulated fisheries (Smith et al. 2010) However, processes such as herbivory and coral expansion that encourage the ecological trajectory towards a self-sustaining reef ecosystem are also evident in the dynamics of the community of benthic encrusting organisms on the reef bottom (Ceccarelli et al. 2011, Fong et al. 2016)

The benthic sessile community of a reef is a complex and competitive arena. The main competitors are often simplified into the following categories: turf, an overarching term for a multispecies, dense, often filamentous mat of algae; crustose coralline algae (CCA) that can promote the growth and recruitment of coral; and scleractinian coral (Steneck & Dethier 1994, Smith et al. 2010, Flower et al. 2016,). Corals, especially highly branched species such as *Pocillopora*, provide structural complexity to a reef ecosystem that attracts diverse and abundant populations of fish and other associated fauna (Stella et al. 2011). Herbivorous coral associates control populations of turf and macroalgae that inhibit the growth and settlement of coral and thus encourage the expansion of the reef (Hixon & Bronstoff 1996, Burkpile & Hay 2010, Humphries et al. 2014).

The most favorable sites for coral reef restoration are where corals previously flourished and are unlikely to settle and grow at a new site under current marine conditions (Rinkevich 2005). Likewise, the best candidates for species to be introduced are those that might not colonize the restoration area unassisted (SER 2004). In Golfo Dulce, an ecologically unique tropical fjord-like embayment on the southern Pacific coast of Costa Rica, historically abundant branching corals in the genus *Pocillopora* have practically disappeared. The initial loss of *Pocillopora* was due the change in the path of rivers that brought fresh water and sediments into the Gulf (Cortés 1991, Cortés & Reyes-Bonilla 2017). In more recent history, coastal development and deforestation around the rivers liberated sediment and resulted in the further destruction of the gulf's reefs and a near

complete loss of *Pocillopora* corals (Cortés 1991). With so few colonies remaining, the population of *Pocillopora* in the gulf could have experienced the Allee effect, a condition of a community where there is not enough population density to sustain sexual reproduction (Allee 1938, Gascoigne & Lipcius 2004). A small number of *Pocillopora* colonies in Golfo Dulce that have demonstrated particular resilience to sedimentation and thermal stress (J. Kleypas pers. comm.) have become the basis of a coral reef restoration effort to preserve and propagate the genus in the Gulf. Given the ecological characteristics of branching corals such as *Pocillopora*, it is reasonable to expect that algal turfs will decrease in area (cm^2) in a site where corals have been transplanted.

Monitoring the health and growth of transplanted corals and changes to the ecosystem around them provides insight into the whether those colonies are functioning ecologically as "ecosystem engineers," and thus can inform future reef restoration efforts. This study aimed 1) to monitor the health and survivorship of transplanted *Pocillopora* colonies in Golfo Dulce, South Pacific Costa Rica, 2) calculate the growth rate of the corals in terms of area, and 3) to describe the effect of the transplantation of those colonies on short time scale succession of the sessile benthic community. This information will aid reef restoration scientists in understanding how the reef community changes following coral colony transplantation.

MATERIALS & METHODS

Study area: Golfo Dulce is a narrow-mouthed embayment (8°27' – 8°45'N, 83°07' – 83°30'W) oriented northwest to southeast between the Osa peninsula and the southern Pacific coast of Costa Rica (Fig. 1). The Gulf has an approximate length of 50km and width between 10 and 15km and covers an area of $680km^2$ with a maximum depth of 200m. The average yearly rainfall in Golfo Dulce is 4,000 to 5,000mm and temperatures ranging from 18º to 35ºC with an average of approximately 26.5ºC. The dry season lasts from December to March with an average rainfall of 100 to 160mm per month. The wet season peaks in October with an average rainfall of 800mm (Cortés 1990). Figure 2 provides rainfall data for the time frame of the study. Figure 3 includes the area-averaged

daytime sea surface temperatures (ºC) from the northern tip of Golfo Dulce for the study period.

Geographically and ecologically, Golfo Dulce is part of the Eastern Tropical Pacific (ETP) marine biological province (Guzmán & Cortés 1993, Glynn et al. 2017). It is often referred to as a tropical fjord due to its anoxic deep waters and bathymetry (Cortés 1990, Wolff et al. 1996). The anoxic waters prevent the energy contained in detritus that sinks to the depths of the gulf from being recycled back into the higher trophic levels of the ecosystem (Wolff et al. 1996). These unique conditions result in a community structure of fish and invertebrates that differs significantly from other marine ecosystems of the Pacific coast of Costa Rica (Alvarado et al. 2014).

Coral Nurseries: The coral colonies used in this experiment were cultivated in nurseries in Nicuesa (Figure 1) as part of the restoration initiative, 1000 Corals for Costa Rica, a project of the NGO Raising Coral Costa Rica and the Center of Marine Science and Limnology (CIMAR) of the University of Costa Rica (UCR). The coral nurseries are tree-shaped structures with central PVC "trunk" and radiating fiberglass "branches" to which the *Pocillopora* colonies area attached with nylon monofilament lariats. These colonies are derived from wild *Pocillopora* from various reefs in Golfo Dulce. The colonies are fragmented by breaking off the tips of the branches with the hands or using a diamond band saw. In order to multiply the number of individual colonies through asexual reproduction (Rinkevich 2005, Horoszowski-Fridman et al. 2015), the nurseries are located between 3 and 5 meters of depth below the water surface and anchored to the sea floor with rope and kept vertical with a floating buoy. The colonies in the nurseries are cleaned of algae each month and their health monitored (T. Villalobos 2019 pers. comm.).

Study organisms: *Pocillopora* is a fast-growing genus (Darling et al. 2012) of branching scleractinian coral and an important reef builder in the ETP (Guzmán & Cortés 1993). *Pocillopora* spp. can be categorized as pioneer species in the establishment of a coral reef due to the rapid growth rate and highly complex structure of corals in the genus

(Clark & Edwards 1994). Thus, *Pocillopora* is a popular genus for coral reef restoration (Boström-Einarsson et al. 2018). This coral is highly important to reef ecosystems due to its complex structure that permits the coral to harbor the greatest diversity of associated organisms of all coral genera (Stella et al. 2011). *Pocillopora* spp. in the ETP are particularly resilient to thermal stress (Manzello 2010), possibly due to their propensity to harbor the more thermally tolerant *Durusdinium glynnii* (formerly "Clade D") zooxanthellae symbionts (Wham et al. 2017). *Pocillopora* may be thought of as a pioneer species and is a good candidate in that their branched skeletons create the conditions that allow other organisms to colonize an area where they previously would not be able (Clark & Edwards 1994, Darling et al. 2012).

The benthic organism and abiotic components of the sea floor in this study were categorized in operational taxonomic units (OTUs). These OTUs included: rubble, sand, bare calcium carbonate ($CaCO_3$), the corals *Pocillopora* and *Porites* spp., dead *Pocillopora* spp. (Dead Coral), unclassified sponge, the macroalga *Caulerpa* sp., Cyanobacteria (Cyano.), articulated coralline algae (ACA), crustose coralline algae (CCA), the bivalve mollusk *Pinna rugosa* and algal turf (Turf).

Transplantation site: Punta Bejuco reef (8°43'39" N, 83°24'29" W) (Fig. 1) was selected as the transplantation site for the *Pocillopora* spp. colonies that were cultivated in underwater coral nurseries near Playa Nicuesa of Golfo Dulce. Punta Bejuco was chosen as a site for transplantation because *Pocillopora* colonies that had been transplanted there prior to this study were healthy and growing (T. Villalobos pers. comm.). The species of corals used in this study were *P. damicornis* and *P. elegans* although they were not distinguished from one another in the experiment.

Pocillopora spp. currently have low rates of natural recruitment in Golfo Dulce (Villalobos 2019 pers. comm.). Small *Pocillopora* colonies are more likely to die as a result of predation (Toh et al. 2014) and out-competition by algae (Kuffner et al. 2006). The coral colonies outplanted at Punta Bejuco had thus been cultivated in coral nurseries from fragments of native colonies in Golfo Dulce to grow the corals to a size adequate for

surviving these challenges. Punta Bejuco consists of three reef-built platforms with steeply sloping edges separated by channels of sand. The reef substrates are mostly made up of dead *Pocillopora* and *Psammocora* rubble. The predominant live coral species are *Porites lobata* (Cortés 1990) and *Porites evermanni* (Boulay et al. 2014). No native living colonies of *Pocillopora* have presently been encountered in Punta Bejuco, but their presence can be inferred from coral skeletons (Cortés 1990).

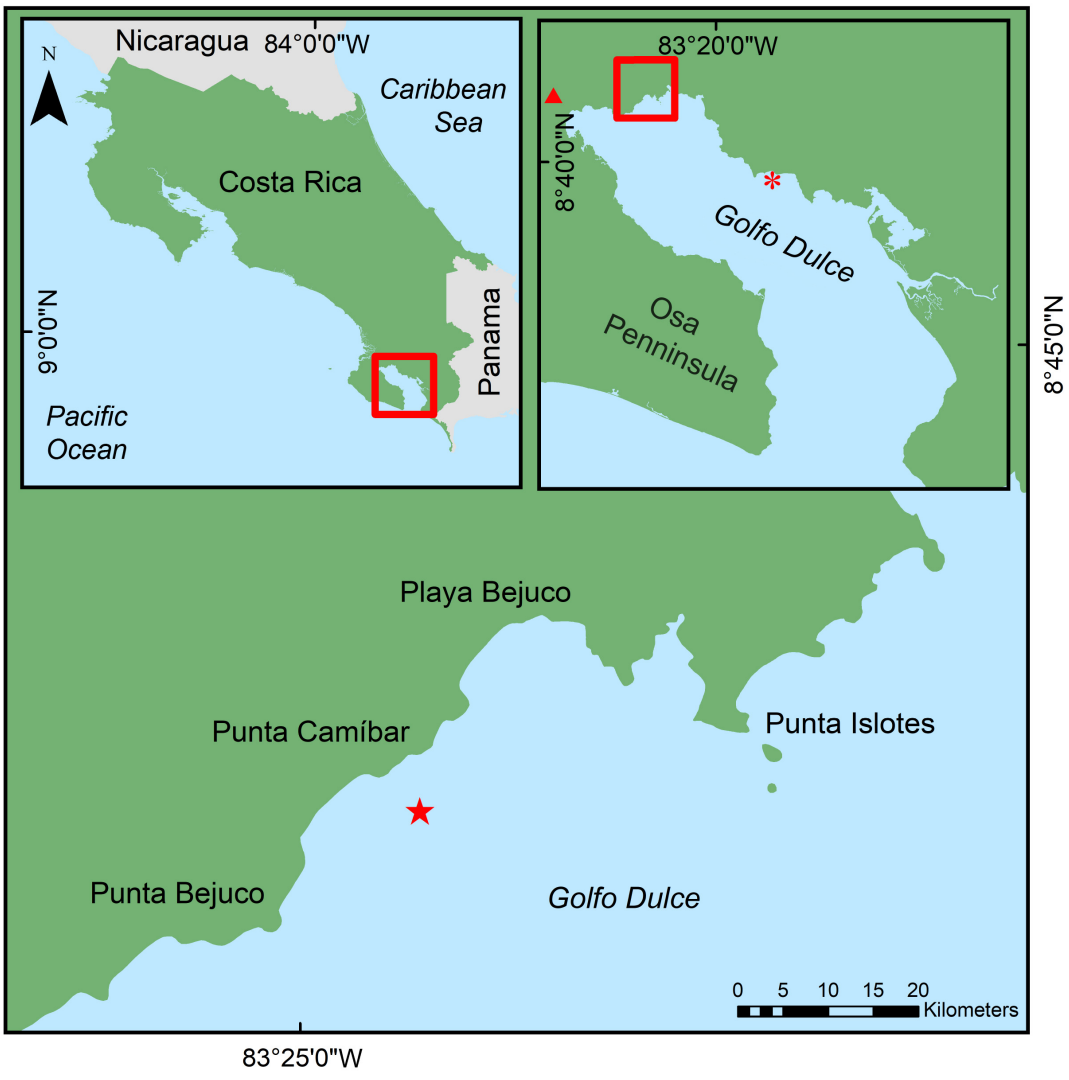

Figure 1. Location of Golfo Dulce within Costa Rica. The transplantation site at Punta Bejuco is marked with a red star and Nicuesa site is marked with a red asterisk in the top-right quadrant. The red triangle in the top-right quadrant marks where the meteorological data were collected (Fig. 2).

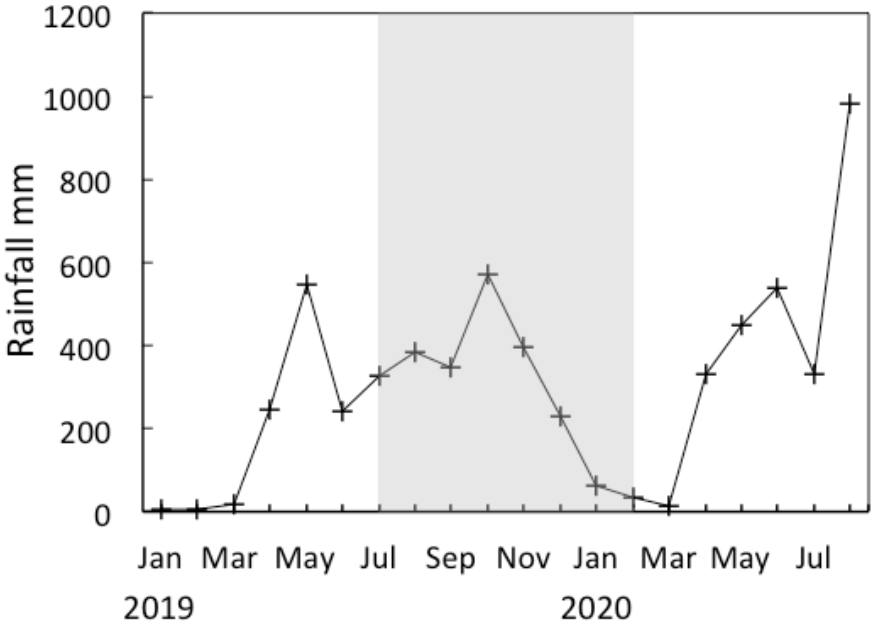

Figure 2. Rainfall (mm month^{-1}) data from Jan 2019 through Aug 2020 from the Instituto Meteorológico Nacional de Costa Rica station, near Golfo Dulce (08º42'03"N, 83º30'49"W, at 80m above sea level). Gray shading indicates the timeframe of the experiment.

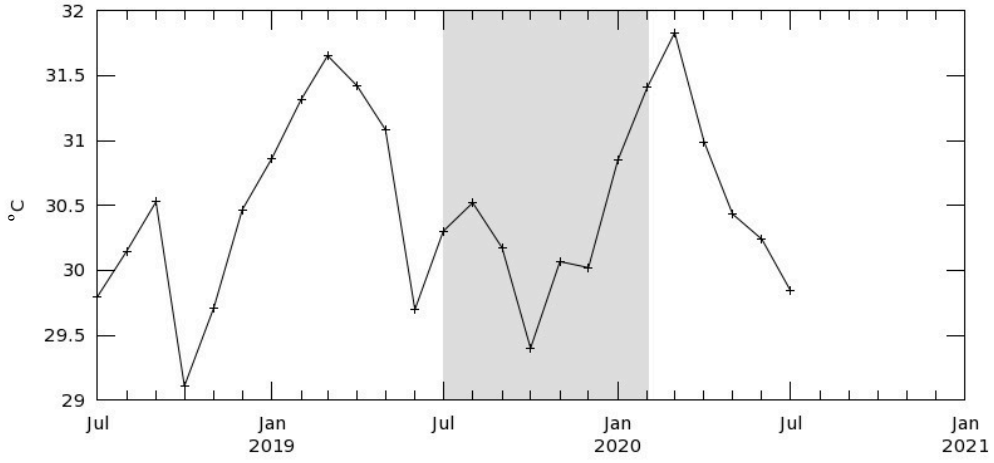

Figure 3. Area-averaged daytime sea surface temperatures from the northern tip of Golfo Dulce from July of 2018 to July of 2020, as derived from the NASA GIOVANNI satellite. Gray shading indicates the timeframe of the experiment.

Out-planting of nursery-grown *Pocillopora* colonies and experimental design: A total of 30 *Pocillopora* colonies were transplanted at the restoration site in Punta Bejuco in July 2019. The colonies were anchored with nylon zip-ties to steel nails, driven into the calcium carbonate substrate in a 6 by 5 colony rectangular arrangement with a distance of 30cm from neighboring colonies. The rectangular transect extended 15cm from the centroids of the fringing nails to measure 1.5 by 1.8m in total (Fig. 4). In order to understand how the presence of the transplanted coral effected the benthic community, a control transect, devoid of coral transplants was designated and monitored using the same methods as the transplantation site. The control transect was located at Punta Bejuco at the same depth as the transplant area in a relatively flat region with a (visually) similar substrate composition. This transect was prepared in the same layout of 30 steel nails as the experimental transplanted transect, but contains no *Pocillopora* transplants (Fig. 4). The control patch contained no corals, living or dead over the course of the experiment. Likewise, the experimental patch never contained *P. rugosa*. Both the experimental and control patches contained negligible areas of sponge and sand.

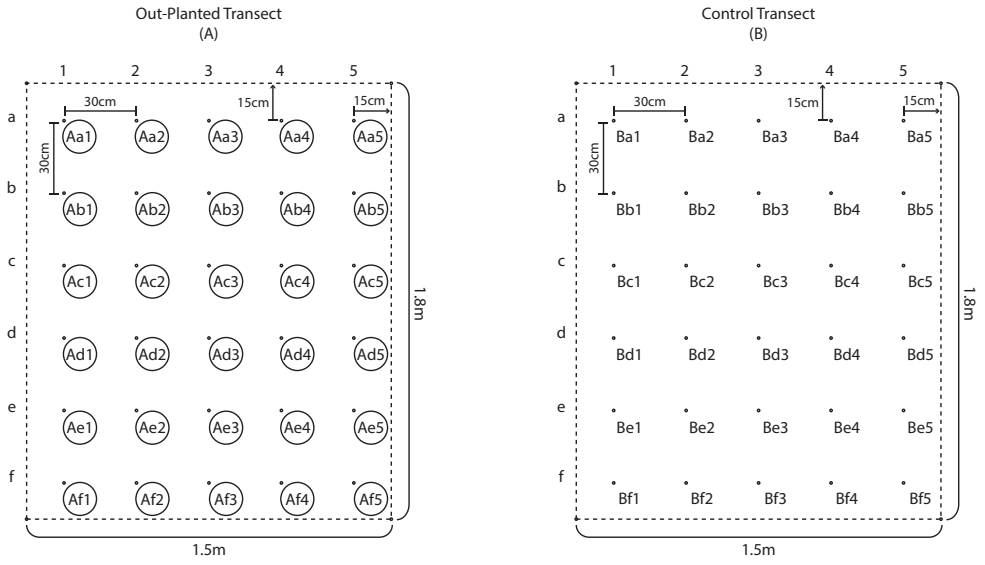

Figure 4. The arrangement of experimental colonies and nails within transects on the sea floor. The black dots represent nails used as attachment points for the colonies or as markers of transect area and quadrat centers. The white circles represent *Pocillopora* spp. colonies. The dotted line represents the edge of the experimental and control transects. Each colony or nail position is given a procedural name so that it can be easily located.

Data Collection and Processing:

Data from the 30 transplanted *Pocillopora* spp. colonies were recorded monthly from July 2019 to February 2020 using photography and observation. Twelve months of data collection were planned, but due to the COVID-19 global pandemic, field trips for data collection were terminated at eight months.

Coral transplant survivorship and bleaching: The bleaching and survival status of each *Pocillopora* spp. colony was recorded each month. Coral could be categorized as bleached, dead or healthy (neither bleached nor dead). If a transplanted coral colony was visibly completely white yet free of macroalgae, cyanobacteria or loose tissues, the colony was recorded as bleached, having lost its symbiotic microalgae. If a colony had tissues visibly separated from the skeleton and/or the exposed skeleton was completely covered in macroalgae or cyanobacteria, the colony was recorded as dead as it no longer had any significant living, growing coral tissue.

In situ photography: To monitor the behavior of the community of sessile benthic organisms, a quadrat around each coral colony was photographed once each month from above using an Olympus TG-5 camera in a proprietary waterproof housing attached to a polyvinyl chloride (PVC) armature that held the camera 40cm above the sea floor. The base of the armature formed a frame measuring 30cm by 30cm and was situated so that each anchor nail was positioned at the center of the base using virtually marked center point on the camera's monitor. For each photograph, the camera was set to "under-water wide mode" with a zoom of 1.1 and set to shoot in RAW and JPEG image file formats. Each image was evaluated using the purpose-made photo analysis software, PhotoQuad (Trygonis & Sini 2012) in order to calculate the area of the transplanted coral colonies and benthic sessile organisms.

Photo Preparation: The JPEG images from the camera were loaded into Adobe Bridge where they were organized and renamed according to the month and colony number or position within the control transect. The images were corrected for "fisheye" distortion from the wide-angle settings of the camera and optical properties of water in the Adobe

Camera Raw image editor. To correct the distortion effectively, the correction was set to the point where the frame in the image was parallel with the gridlines in Camera Raw. The distortion correction was set to -31 for all of the images.

Quadrat assignment: Once loaded into the PhotoQuad application, distances in the distortion-corrected images of each coral were calibrated individually by selecting the "calibrate" toggle and dragging the ends of the calibration bar to the bottom inner edge of the T-joints on the left and right of the PVC frame. The length of the calibration bar was then set to 30cm, the true distance between the supports of the PVC frame (Trygonis & Sini 2012).

Coral area measurement: The quadrat of each image was assigned manually using the "manual detection" toggle in the PhotoQuad application. The quadrat was defined by dragging the points supplied by the application to the inner corners of PVC frame to draw straight lines on the inner borders and around the joints of the PVC frame. The area of the coral colonies parallel to the substrate was measured with the "region of interest" (ROI) method in PhotoQuad (Trygonis & Sini 2012). In this method, the border of the living coral tissue (tissue that was neither visibly separated from the skeleton nor covered in macroalgae nor cyanobacteria) was traced and assigned as *Pocillopora damicornis*. The data from PhotoQuad were then exported as comma-separated values (.csv) for analysis in the statistical software 'R' (R Core Team 2013). The "SPECIES Area Total cm2 (*sic*.)" PhotoQuad output variable was used for analysis. This value was defined by the following formula:

$$Area_{cm^2} = A_{pix} \times {f_{clb}}^{-2}$$

Where:

A_{pix} : is the number of pixels enclosed within ROI;

f_{clb}: is the calibration factor of the image based on the distance between the inside edges of the PVC frame in the image.

Sea floor coverage measurement: The benthic community of the experimental transects was defined as the area of each encrusting organism OTU or abiotic benthic component within the quadrat. The area of each of the encrusting organisms was measured in PhotoQuad using the *grid method* (Trygonis & Sini 2012). A grid was drawn over the quadrat with the *horizontal grid* field set to 150 pixels, equivalent to a 2250 pixel cell. Grid cells were selected when positioned over an encrusting organism. When the grid cells fell over the borders of the encrusting organisms, the fraction of the cell occupied by the encrusting organism was estimated and added to other occupied fractions of grid cells. When the fractions added up to one (1), a grid cell was selected. Selected grid cells were assigned to the OTU they covered. The data consisted of the area covered by the OTU in cm^2, pixels, and the proportion of the quadrat occupied by that OTU. These data were imported into R for analysis (R Core Team, 2013).

Outliers: Quadrats where the coral colony extended beyond the PVC frame in the image were removed from the dataset because they could not be measured accurately by the photographic method. This resulted in the removal of two (2) quadrats from the experimental patch data set each month.

Statistical analysis:

Growth rate: In order to estimate the rate of growth in area (cm^2) of the corals over the eight months of the experiment, the average area (cm^2) of each *Pocillopora* colony were plotted each month with line-of-best-fit from linear regression. The slope of the line-of-best-fit was the growth rate of the colonies in $cm^2\ month^{-1}$.

Comparison of experimental and control: Non-metric multidimensional scaling (nMDS) from Bray-Curtis similarity matrices was used to detect differences in the sea floor community composition in the initial (July 2019) and final (February 2020) months in the experimental and control patches. These similarity matrices were built using the areas of each OTU in each of the photoquadrats. The area values of OTUs in each patch were square-root transformed in order to ensure that OTUs that occupied small proportions of each photoquadrat were would not “drowned out” by the OTUs that occupied the

majority of the photoquadrat area and could be compared on the same scale. These area values excluded *Pocillopora* colonies, dead coral and artificial structures (anchor nails, tags and zip ties) to ensure that any differences in benthic community resulted from the presence of transplanted coral rather than simply detecting the growth of the transplants themselves. ANOSIM was performed on the nMDS data to quantify the separation and significance of any difference between initial and final benthic communities. SIMPER was performed to identify which OTUs contributed most to any differences that were detected. Construction, visualization and analysis of nMDS were performed in PRIMER 7 (Clarke & Gorley 2015).

Temporal changes in sessile benthic community: Changes in the area of OTUs in each patch over time were estimated with linear regression in R (R Core Team, 2013). In the experimental patch, measurements from quadrats where the coral colony had died were analyzed separately from those that survived the 8 months of the experiment. Corals were identified as dead when they were devoid of living coral tissue and covered with algae. The quadrats were separated in this manner in order to see if temporal patterns in the abundance of benthic organisms were different when the coral transplant had died.

RESULTS

Coral Survivorship and bleaching: In the eight months following transplantation (July 2019 – February 2020), five of the 30 (17%) colonies died; three (10%) died in October (four months from planting) and two more in November (7%) (Table 1). The colonies experienced two episodes of bleaching: the first, in August and September 2019 (13 and 52% of the original 30 colonies, respectively) and the second, in January and February 2020 (10 and 67% of the original 30 colonies, respectively) (Table 1). At the end of the experiment, 5 colonies had died and of the remaining 25 living colonies, 20 were bleached and 5 were healthy.

Table 1. Total number of transplanted *Pocillopora* spp. colonies that were bleached, dead, or healthy (neither bleached nor dead) each month in the experimental patch in

Golfo Dulce. The number of colonies reported (n) varied from month to month due observational challenges.

Month	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Total *n*	30	24	29	30	30	30	29	30
Total Bleached	0	3	15	0	0	0	3	20
Total Dead	0	0	0	3	5	5	5	5
% Bleached	0%	13%	52%	0%	0%	0%	10%	67%
% Dead	0%	0%	0%	10%	17%	17%	17%	17%
% Healthy	100%	87%	48%	90%	83%	83%	73%	16%

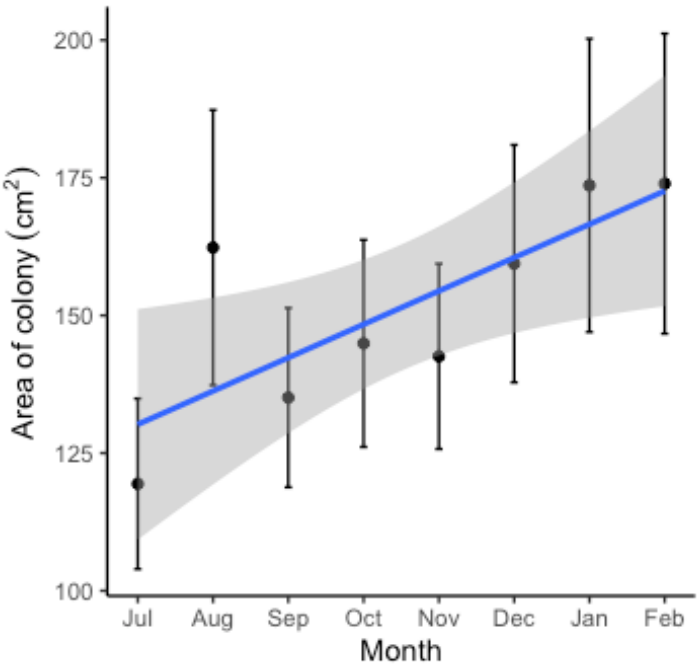

Figure 5. Monthly average area (cm^2) of transplanted *Pocillopora* colonies in the transplanted site in Golfo Dulce, with standard deviation and line-of-best-fit from linear regression (line-of-best-fit equation: Area of colony = 121.3 + 6.47(no. months following transplantation)); the gray area represents 95% confidence intervals.

Growth: The area of live *Pocillopora* spp. colonies increased significantly (linear regression: $p < 0.05$, $R^2 = 0.01798$) from an average of 119.4 ± 72.6 cm^2 in July 2019 to 175.9 ± 113.0 cm^2 in February 2020, a 68% growth in area on average from initial to

final months. The change in area of the *Pocillopora* spp. transplants was calculated to be 6.47cm^2 month^{-1} based on linear regression ($R^2 = 0.018$, $p < 0.05$) (Figure 5).

Change in the benthic community composition: The composition of the benthic community differed significantly between July 2019 and February 2020 in the experimental patch (ANOSIM; $p < 0.01$, $R = 0.147$) but not in the control patch (ANOSIM; $p > 0.05$, $R = 0.031$) (Figure 6). Based on SIMPER analysis, the main contributors to the difference in the benthic community in the experimental patch after 8 months following coral transplantation were turf, CCA and Cyano.; contributing to 34, 27, and 37% of the difference respectively (Table 2).

Table 2. Similarity of assemblages of benthic organisms in the experimental patch at Punta Bejuco, Golfo Dulce and OTUs with the greatest contribution as the result of SIMPER.

Group	**Average Similarity/Dissimilarity**	**OTU**	**% Contribution**
February	84.68% similar	Turf	90.42
July	81.03% similar	Turf	90.47
February vs. July	19.45% dissimilar	Turf	33.67
		CCA	27.32
		Cyano.	26.39

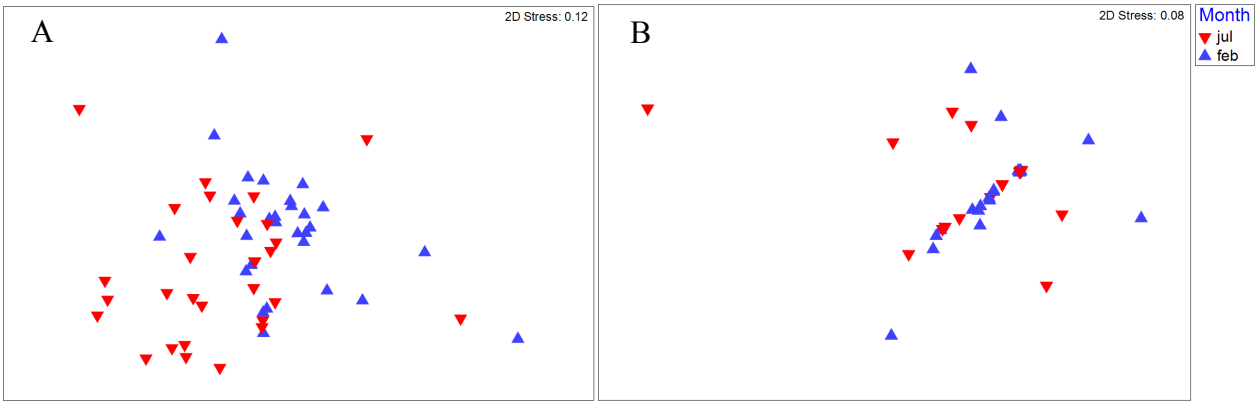

Figure 6. Non-metric multidimensional scaling of the benthic communities around *Pocillopora* spp. transplants in the experimental patch (A) and the transect-marking nails in the control patch (B) excluding live or dead coral and artificial structures in July 2019 versus February 2020 in Punta Bejuco, Golfo Dulce.

Change in area and coverage of the benthic communities: In the experimental patch, the areas of turf, cyanobacteria, dead coral and CCA changed significantly over time (linear regression: $p<0.05$) in the 24 quadrats where the colonies remained alive throughout the experiment. The coverage of turf decreased by an average of 11.1 cm^2 per month and the coverage of cyanobacteria, dead coral, and CCA increased by an average of 4.2, 1.1, and 3.1 cm^2 per month respectively. In the four quadrats where corals died, the areas of *Pocillopora* spp., cyanobacteria, and dead coral changed significantly over time (linear regression: $p < 0.05$). The area of living *Pocillopora* spp. decreased by 29.1 cm^2 per month and the areas of cyanobacteria and dead coral increased by 32.2 and 24.5 cm^2 per month respectively (Figure 7). In the control patch, the areas of OTUs remained constant and none changed significantly over time from their initial values.

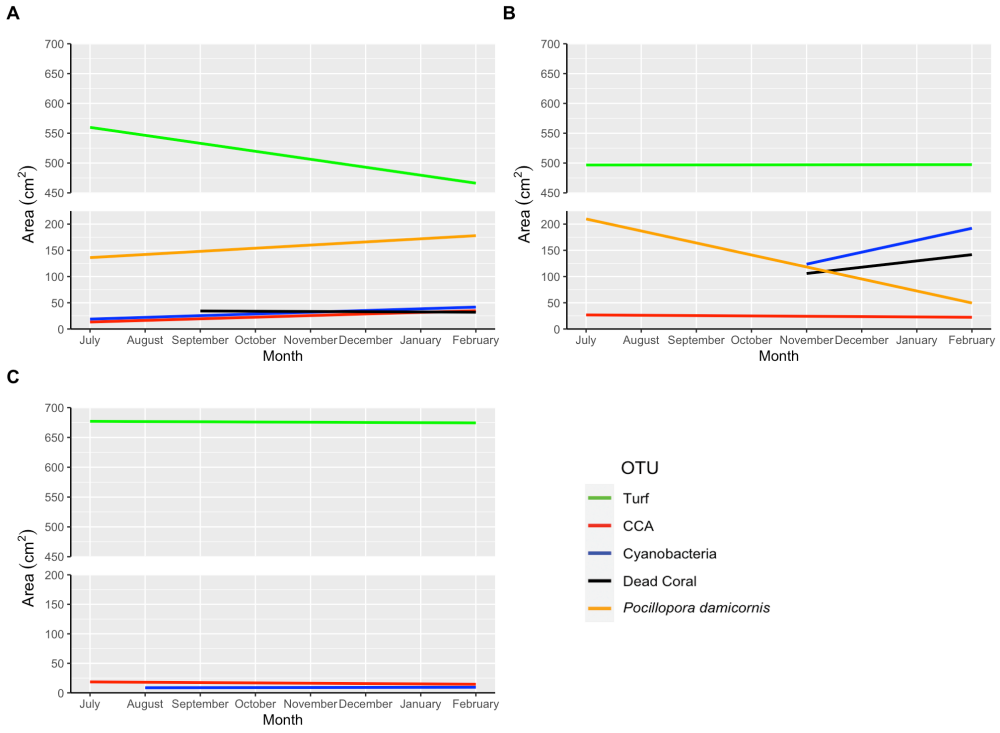

Figure 7. Areas (cm^2) of benthic sessile organisms over time in the quadrats where corals in the experimental patch survived for the length of the experiment (A) or died before the end of the experiment (B) and in the control patch (C) at Punta Bejuco, Golfo Dulce.

The OTU that decreased the most in coverage was *Pocillopora* spp. in the "dead" coral quadrats with a decrease from 23.55 to 2.52% of the total coverage. In the "dead" quadrats, the coverage of cyanobacteria and dead coral increased dramatically as well from 0 to 20.20 and 0 to 15.20% respectively, taking up more of the quadrats than the initial coverage of the *Pocillopora* spp. transplants by 11.85%. The average coverage of live *Pocillopora* spp. increased by 7.95% from 16.89 to 24.84% in "live" coral quadrats. In the control patch, no OTU changed more than 1.5% in average coverage of the quadrats (Table 3). No sand or sponge was present in the control nor experimental patches.

Table 3. Average percent proportions (SD) of the quadrat area occupied by benthic organism OTUs in the initial (July) and final (February) months of data collection at Punta Bejuco, Golfo Dulce. Averages are grouped by the patch and status of coral outplants as follows: Live – quadrats in the experimental patch where the coral survived the entire 8 months of the experiment; Dead – quadrats in the experimental patch where the outplants died at some point over the course of the experiment; Control – quadrats (Cons) in the control patch.

Status	*n*	**Rubble**	**ACA**	**Bare $CaCO_3$**	***Pinna rugosa***	***Pocillopora* spp.**	**Caulerpa spp.**	***Porites* spp.**	**Turf**	**Cyano.**	**Dead Coral**	**CCA**
Live Jul Avg	23	0.0(0.0)	0.0(0.0)	0.1(0.5)	0.0(0.0)	16.9(10.6)	0.3(0.7)	0.5(2.4)	80.5(10.5)	0.18(0.9)	0.0(0.0)	1.6(3.1)
Live Feb Avg	17	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	24.8(13.2)	0.1(0.4)	0.8(3.3)	65.4(11.7)	3.6(4.2)	1.0(2.7)	4.2(3.8)
Dead Jul Avg	4	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	23.6(7.3)	0.0(0.0)	0.0(0.0)	73.5(6.0)	0.0(0.0)	0.0(0.0)	2.9(2.5)
Dead Feb Avg	4	0.0(0.0)	0.2(0.3)	0.0(0.0)	0.0(0.0)	2.5(2.9)	0.0(0.0)	0.0(0.0)	60.2(12.8)	20.2(9.4)	15.2(7.4)	1.7(1.5)
Cons Jul Avg	21	0.2(0.9)	0.0(0.0)	1.1(3.7)	0.1(0.3)	0.0(0.0)	0.0(0.2)	0.0(0.0)	97.1(6.2)	0.0(0.0)	0.0(0.0)	1.5(2.7)
Cons Feb Avg	29	0.0(0.0)	0.0(0.0)	0.2(0.9)	0.1(0.5)	0.0(0.0)	0.0(0.0)	0.0(0.0)	98.5(2.2)	0.0(0.1)	0.0(0.0)	0.9(1.6)

DISCUSSION

Coral health and survivorship:

Over the course of eight months of the study, there were two bleaching events and 17% of the transplanted colonies died. The first event occurred in August and September 2019. High rainfall in these two months typically causes significant run-off and sedimentation in the gulf as sediment is washed in from the surrounding rivers that feed into Golfo Dulce. This sedimentation was one of the primary causes for the loss of *Pocillopora* spp. colonies in the Gulf in the 1960's-80's (Cortés 1990). Sediment settles on the coral

colonies, blocking sunlight, and stimulating the production of excess mucus, causing stress and bleaching (Philipp & Fabricius 2005). Alternatively, bleaching could have occurred as the result of the stress of the transplantation process, an event observed in other restoration efforts (Forrester et al. 2012, Cunning et al. 2014).

The second bleaching event occurred in January and February 2020. These months correspond to the dry season when water temperatures are elevated and the sea surface temperature averaged 31.2°C (figure 3) during January and February 2020. Although temperature tolerance can vary significantly within corals of the same species from different regions (Coles et al. 1976), corals in the ETP have been reported to bleach when sea surface temperatures exceed 30ºC (Podestá & Glynn 1997). In general, corals often do not recover following severe bleaching. The majority of the bleached corals at the transplantation site regained some of their original color and grew significantly although none of the coral transplants regained the color they had in the first month of the experiment immediately following transplantation (personal observation).

Given that none of the *Pocillopora* transplants survived longer than 9 months following transplantation (J. Kleypas pers. comm.), a different location and/or different environmental conditions should be considered for future restoration efforts in Golfo Dulce. The relatively shallow depth of the corals at 3m may have offered little protection for the corals from higher temperatures near the water surface, or higher light intensity; a problem encountered in other coral transplantation efforts (Lohr et al. 2016). It is generally recommended to transplant corals at the depth they are encountered naturally in the area. In Golfo Dulce, *Pocillopora* is encountered from 3 to 5 m below the surface (Cortés 1990) although most of the native colonies that provided fragments for the nursery were deeper, up to 8 m below the surface (J Kleypas, pers. comm.). As of October 2020, almost all of the nearby coral transplants that were not part of the experiment and which were positioned deeper at 5.6m survived the bleaching event in January and February 2020 (T. Villalobos 2020, pers. comm.).

Coral growth:

Despite the two bleaching events and non-ideal transplantation site, the corals grew at a rate where on average the colonies could double in area from their initial size at transplantation in 18.5 months if they continued on the same trajectory as the first 8 months. Other research suggests that the average sizes of the *Pocillopora* spp. colonies in the experimental patch were in a range of sizes that are likely to grow but less likely to survive over the course of a year (Kodera et al. 2020). This practical information could be very useful for determining the size of coral transplants to use for the purposes of restoration.

Temporal change in the sessile benthic community following transplantation:
The increase in area of live coral, CCA and Cyanobacteria and decrease in area of algal turf in the experimental patch following the transplantation of *Pocillopora* spp. colonies suggests that the coral colonies affected the succession of the sessile benthic community. Corals, especially structurally complex, highly branched forms such as *Pocillopora*, attract associated fauna (Lewis 1986, Smith et al. 2010). Herbivorous coral associates contribute to the health and resilience of coral reefs by preventing algal succession from progressing from a state dominated by early successional CCA which do not compete with corals and fleshy, leafy algae which impede growth of corals (Hixon & Bronstoff 1996, Burkpile & Hay 2010, Humphries et al. 2014).

The lack of change in the coverage of sessile benthic organisms in the control patch further supports the conclusion that coral had an effect on the trajectories of succession of the benthic community. It can differing trends in the area of turf between experimental and control patches may be the result of greater herbivory in the experimental patch from herbivores attracted to the structural complexity of the transplanted coral (Lewis 1986, Smith et al. 2010, Salinas-Akhmadeeva & Reyes Bonilla 2017). Conversely, the area of algal turf in the control patch remained constant and high because it is less structurally complex and thus attracts fewer herbivores (Lewis 1986).

Over the entire experimental patch and particularly in quadrats where corals had died, there was a strong increase in the coverage of cyanobacteria. In quadrats where the coral

transplants had died, the area of cyanobacteria, along with dead coral tissue had almost entirely replaced live coral by the end of the experiment. Cyanobacteria flourish in high temperatures and could have contributed to the ultimate death of the coral colonies following bleaching by smothering the weakened coral and benthos (Kuffner et al. 2006, Ford et al. 2018,). Cyanobacteria may produce toxins that could deter herbivores that would otherwise control macroalgal populations (Leão et al. 2012, Ford et al. 2018). It is important to understand the characteristics and processes that occur when coral colonies die because it can provide clues and warnings when monitoring the health of reefs and implementing management responses (Goatly et al. 2016). This could be a problem for future coral transplantation in the zone as the environmental conditions in the shallow reefs of Golfo Dulce could favor the growth of Cyanobacteria.

The decrease in algal turf in the experimental patch from ~80 to ~65% is comparable in magnitude to the decrease in turf coverage at the coral reefs of Coco Island following 20 years of reef recovery after the 1982-83 ENSO event (Guzmán & Cortés 2007). Coral coverage recovered from an average of 2.99±0.25 to 14.87±6.78 (average±SE)% at Coco Island, a similar magnitude to this study with the experimental patch containing a negligible coverage of coral before transplantation and upwards of 24.8% coverage 8 months following transplantation. Although the study at Coco Island was conducted at a much larger spatial and temporal scales, and under much higher conservation standards than in Golfo Dulce, similar processes may account for the decrease in turf coverage albeit over a much shorter time period in this study. Restoration, together with other coastal management actions, could increase the survivorship of the corals and the recovery of the reef and their ecological functions.

Limitations:

There were limitations to the experiment in terms of scope, methodology and background information. The first is the short time frame of the experiment. Although one of the shortest times reported for succession to reach a climax in a coral reef ecosystem is approximately 10 months (Matsuda 1988), the process of succession on coral reefs

generally takes much longer than the timeframe of this experiment (Karlson 1999). Over a longer timeframe, further evidence of succession and its associated ecological processes could be detected. Also, following the ultimate bleaching and death of the coral transplants, a longer experiment could explore how succession of the sessile benthic community changes following the death of corals, a process that was beginning to occur with the increase in cyanobacteria.

The experiment was also limited by the spatial scale of observations. Photoquadrats were positioned directly over the transplanted coral colonies in the experimental patch, resulting in a bias toward greater coral coverage. An experiment using larger quadrats could capture the dynamics of the sessile benthic community at the scale of a reef rather than a colony. The differences observed in the dynamics of the benthic communities in the experimental and control patches may be the result of methodological artifacts. The observed decrease in turf area may be the result of the coral transplants taking up more area of the photoquadrats as their branches grow up and over the sea floor. The branches could be covering turf in the image that may still be present around the bases of the colonies and thus, reduce their apparent area.

CONCLUSIONS

Ecological succession is intrinsically important to restoration efforts. Environmental and ecological conditions can direct the succession of a reef towards alternate stable states (Smith et al. 2010). Under current environmental conditions, many coral reefs tend to follow a successional trajectory that favors the dominance of algae and other organisms that inhibit the recruitment and growth of corals (Rinkevich 2005, Pandolfi et al. 2005). Coral restoration through transplantation is an increasingly common intervention that is used to accelerate the process of succession to favor the growth and persistence of coral reefs.

The transplantation of *Pocillopora* spp. colonies influenced the succession of a sessile benthic community of a restoration site in Golfo Dulce, as evidenced by the growth of

organisms that promote coral growth and the decline of those that compete for space and resources with corals. Additionally, following transplantation, these shifts in the sea floor composition favored a successional trajectory that promotes the growth of coral and may improve the reefs' resilience to future disturbance and the acceleration of ecosystem recuperation. Transplantation and growth of *Pocillopora* spp. colonies corals modified the local ecosystem around the colonies to promote further coral reef growth and development. These results suggest that the transplantation of *Pocillopora* spp. could improve the outcomes of coral restoration efforts by changing the trajectory of succession. However, this must be carried out in conjunction with watershed management to reduce sedimentation in the area and associated stress to the corals. Likewise, for an ecosystem management of fisheries that favors the recovery of herbivorous fish populations that help control other more aggressive benthic competitors of the substrate such as algal turf. A similar experiment conducted over longer temporal and larger spatial scales may reveal if successional trends continue or change.

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